The Journal of Laboratory and Clinical Medicine

VOL. 27 MAY, 1942 NO. 8

CLINICAL AND EXPERIMENTAL

THE ORIGIN AND NATURE OF THE CABOT RING BODIES OF ERYTHROCYTES*

EMIL MARO SCHLEICHER, MINNEAPOLIS, MINN.

INTRODUCTION

CABOT[1] in 1903 enriched the literature with an unique contribution. He described red and blue staining circular and twisted bodies within basophilic, polychromatic, and infrequent orthochromatic erythrocytes, first brought to his attention by one of his associates in a case of lead poisoning and thereafter observed by himself in the anemic blood of patients suffering from pernicious anemia, lead poisoning, and lymphatic leucemia.

The dissertation aroused much interest for a twofold reason: first, no previous mention of such bodies had been made; and second, Cabot's hypothesis that the ring bodies may represent "nuclear remnants" was diametrically opposed to the tinctorial behavior of the structures.

When this paper went to press, the literature contained no feasible explanation for the peculiar affinity of these supposed nuclear derivatives for cytoplasmic stains; no actual proof withstanding critical analysis that the ring bodies are nuclear remnants, and no convincing data to show that they are laboratory creations.

It is the purpose of this communication to show that the Cabot ring bodies are denatured protein configurations, i.e., they are actually laboratory products. The phenomenon occurs in specifically altered cells only, i.e., in erythrocytes and certain erythroblasts that have undergone lysis. This statement is supported by two facts: (a) the creation of numerous Cabot ring bodies at will, in blood samples taken from cases with pernicious anemia in severe relapse, acute lead

*From the Hematological Laboratory, Institute of Anatomy, University of Minnesota.
Received for publication, May 17, 1941.

poisoning, terminal lymphosarcoma, acute malaria, terminal lymphatic leucemia, congenital hemolytic icterus during hemolytic crisis, erythroblastosis fetalis, and a case with generalized Hodgkin's disease; and (b) the production of simulacrums in normal, pathologic human erythrocytes, and the creation of analogous structures with egg white by special methods.

For those who are not familiar with the problem at hand the major articles and theories will be presented briefly. Cabot's dictum that divided students of the subject into two groups, those who advocate that the ring bodies are remnants of the nuclear membrane, and those who claim that they are artifacts, is cited first:

"There is no reason to believe them a mark of cellular degeneration . . . . they have some connection with the nucleus formerly possessed by the cell that contains them seems to me not unlikely . . . . their shapes correspond for the most part very well with those of nuclear figures . . . . the best hypothesis, therefore, that I can suggest to account for these appearances is that they represent nuclear remains . . . . the significance of their red color with Wright's, a stain which never stains any nucleus red, is not clear to me . . . . have these figures any practical significance? Not so far as I know."

Thus Cabot, while very conservative and noncommittal regarding the nature of the bodies, precipitated, by this very approach to the phenomenon, an extensive literature dealing with the mode of origin of the structures bearing his name.

Among the investigators who believe the rings to be identical with the nuclear membrane are Schleip,[2, 3] Pappenheim,[4-6] Sluka,[7] Alder,[8] Ferrata,[9, 10] Ferrata and Viglioli,[11] Juspa,[12] and Jordan, Kindred, and Beams.[13] Ferrata's hypothesis: "The Cabot ring bodies are the peripheral residue of the nucleus and are produced by vacuolization of the central mass" has become a prevailing view, in spite of his observation that generally the rings stained with pyronin and only an occasional one showed affinity for methyl green, specific for nuclear derivatives. In Ferrata and Viglioli's paper, the concept was enlarged by the authors' statement that "the Cabot ring bodies are morphologically identical with the peripheral part of the nuclear membrane. They are always a sign of a typical pathological maturation of the erythroblasts, the ring bodies occur only in severe anemias." Juspa proposed the following theory: "The ring bodies are not typical nuclear chromatin; they represent nuclear remnants and in all probability are the membrane left behind through pathological endonuclear vacuolization processes and karyolysis." Jordan and coworkers concluded: "Moreover, the genuine Cabot rings seem clearly to represent persistent nuclear membranes."

Among those who proposed that the Cabot rings are artifacts is Demel,[14] who assumed that "the Cabot rings represent the boundary zone between an inner area of more rarefied hemoglobin and an outer more condensed area." Demel was working with normal blood, using a stain (Neo-Giemsa) formulated by Dr. Polettini of Pisa. His theory is refuted, however, by the fact that the rings can be produced after the hemoglobin has been laked.

Russow,[15] commenting on Sluka's paper, pointed out that if the Cabot rings are nuclear remnants, they should be seen in the bone marrow, and their appear-

ance should obviously be more frequent in this organ. He concluded: "the rings are most likely the expression of unusual drying processes of the anemic blood, since the bodies have only been observed in dried peripheral blood preparations."

The neutral side in the controversy is represented by Naegeli,[16] who summarized the literature as follows: "Cabot rings are generally considered nuclear membrane remnants, absent in erythroblasts and the blood forming organs; they are a pathologic phenomenon of nuclear dissolution." Schilling[17] states: "It is not certain that the Cabot-Schleip rings are nuclear remnants." In another publication[18] he records seeing Cabot rings in normal erythroblasts. He further suggests, according to Schröder,[19] that the rings may represent the contour of the "glass body" of the erythrocyte. Isaacs,[20] in Downey's *Handbook of Hematology*, informs us that Cabot rings have been found in cells with perfectly intact nuclei and entirely separate from the nuclei. Isamboulas and Malikiosis[21] present in their article a photomicrograph showing a Cabot ring in the protoplasm of a normal erythroblast obtained by sternal aspiration from a patient with typhus. They come to the conclusion that their observation is "opposed to the prevailing view."

That Cabot rings occur in erythroblasts has been ascertained from the literature cited and from my own observations, but their presence is rare indeed. Over a period of many years I have seen only two specimens. The infrequency is especially brought out by the fact that no clear-cut example was noted by Dr. Hal Downey, in his large collection of preparations in the field of hemopathology. The erythroblast shown in Fig. 14 was found in one of the smears made from the peripheral blood of the case with generalized Hodgkin's disease mentioned above. The only other one was seen in the bone marrow of a patient with severe relapsed pernicious anemia, having about 650,000 red blood cells per c.mm. A probable reason for the scarcity of the presence of Cabot rings in erythroblasts is given elsewhere in this paper.

In over 2,150 bone marrow aspirations,[22, 23] covering practically all well-known hematologic disorders (mindful of the admixture of sinusoidal blood with the bone marrow specimen), I have in no instance observed a greater number of Cabot rings in the bone marrow preparations than were seen in those made from the peripheral blood. No exception was the case with acute sickle-cell anemia,[24] where in the "usable area"[25] the erythroblasts numbered eighty-four among one hundred leucocytes, but only four erythrocytes with Cabot rings were observed per 180 micrometer squares.[26] The scarcity of Cabot ring bodies seemingly is the rule rather than the exception in severe erythroblastic anemias and toxic anemias associated with erythroblastemia and icterus (leading by their very nature to severe disturbances of the denucleation process), where one would expect Cabot ring bodies to be plentiful in the bone marrow. As already pointed out, this is not the case. Furthermore, dark-field examinations have never revealed that Cabot ring bodies are preformed structures, i.e., nuclear membranes. The hypothesis that the Cabot ring bodies are neither nuclear remnants nor a sign of pathologic denucleation processes, is strengthened by the observations cited in this paragraph.

Structures similar to those described by Cabot have been produced by various investigators.[27, 28, 38, 45-47] These simulacrums were obtained by exposing erythrocytes to a hemolytic system. It may then be assumed that the creation of the ring bodies depends upon the degree of lysis an erythrocyte or erythroblast has undergone in vivo or in vitro, induced by a specific agent. Thus a plausible reason is advanced for the appearance of these bodies in disorders of the hematopoietic organs associated with severe anemias, and with or without elevated icterus.

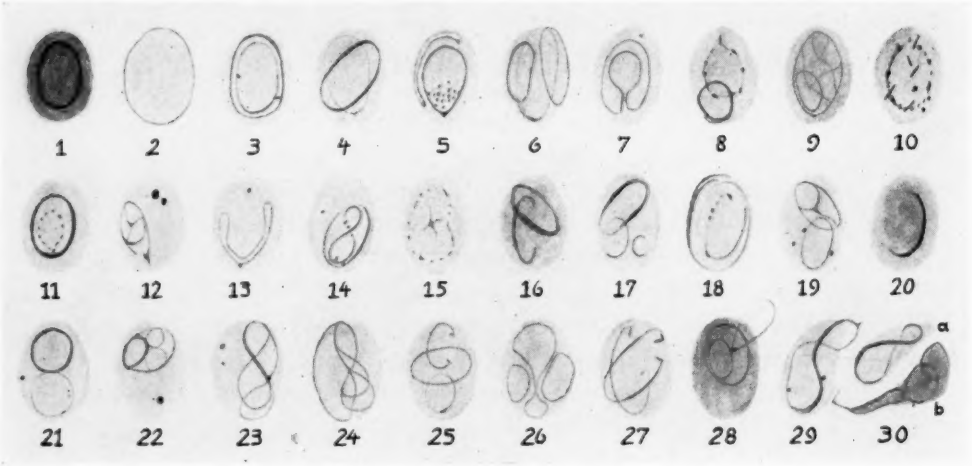

Plate I.
Basophilic and polychromatic erythrocytes showing "denatured protein configurations" (Cabot ring bodies).

THE ORIGIN OF CABOT RING BODIES

A blood smear was presented to me by Dr. R. H. Jaffé* in which he had observed Cabot ring bodies showing an unusually brilliant red hue, as well as width and flatness of the ring bodies. The smear was made from the peripheral blood of a moribund patient with generalized Hodgkin's disease. Figs. 1 and 2*a* show examples of the bodies seen by Dr. Jaffé. Examination of the preparation revealed that there were only three erythrocytes with Cabot ring bodies per 180 micrometer squares.[26] Erythroblasts were numerous in this blood, and many cells showed pathologic denucleation. A severe anemic state was present: red blood cells, 1,020,000, hemoglobin, 3.0 Gm., icterus index, 15 (Wintrobe), and a reticulocytosis of 18.4 per cent; occasionally a reticulocyte with ring bodies. Figs. 2 to 6. As the number of ring bodies was too small to trace their origin, peripheral blood was defibrinated and permitted to sediment in a Wintrobe hematocrit tube. The procedure was designed to gather more specifically altered red cells assuming that hemolyzed cells tend to form the uppermost layer of the blood column. While smears made from the top layer yielded a larger number of ring bodies, I was unable to trace intermediate stages between the nuclear membrane and the Cabot ring bodies.

*Chief pathologist of Cook County Hospital, Chicago, Ill.

An experiment was then set up that conformed to Russow's theory. Peripheral blood was spread on glass slides, placed in a slanting position while drying, allowing the plasma to accumulate near one end of the blood films, and thus delaying the drying process. The control slides were made in the standard manner, rapidly whipping them through the air to accelerate drying. On examination of the preparations, the following observations were made: Many circular and twisted Cabot ring bodies were seen in the area where drying was delayed, whereas the opposite area showed only occasional bodies. The control slides revealed about the same number of ring structures as noted in the rapidly drying area of the slanting preparations. This was definite evidence that the ring bodies were created by delaying the drying process. Plate I shows some of the Cabot ring bodies produced by the slanting method.

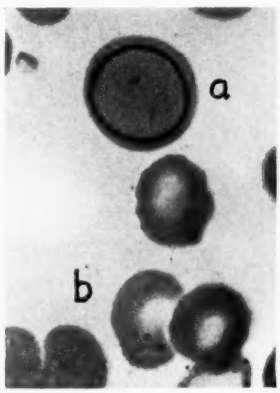

Fig. 1.

Fig. 2.

Fig. 1.—Mr. A. K. Generalized Hodgkin's disease, peripheral blood. *a*, Orthochromatic erythrocyte with a typical Cabot ring body. Note that this denatured protein body has a considerable width and appears to be a flat structure within a uniformly stained cell. *b*, Markedly lysed erythrocyte with a fine blue ring.

Fig. 2.—Mr. A. K., peripheral blood. *a*, Polychromatic erythrocyte with a figure-eight body and a bar at the crossing point. *b*, Reticulocyte showing a figure-eight body intersecting an incomplete ring structure. Two globules and an aggregation of protein at the cell periphery (at arrow).

The formation of the figure eight may be as follows: two rings form simultaneously in the same plane and flow together, or rings unite from adjacent planes, or a ring retracts rapidly and overlaps.

At no time were the angles great enough to encourage concentrations of red blood cells which might account for the increased number of ring bodies at or near the edge of the blood smear. An estimation of the erythrocyte number with the dry chamber method[26] showed an error of from 4.2 to 5.4 per cent for the preparations made by the slanting method, in comparison with the counting chamber number. While in this particular blood the number of ring bodies could be varied at will, this could not be accomplished to the same extent in all blood samples taken from cases with severe anemias, showing one to several ring structures per 180 micrometer squares. In this test series the serum icterus ranged from 2 to 280. When no ring bodies were seen in the routine smears,

their creation by the slanting method frequently met with failure. Fig. 3 shows the development of a Cabot ring body as noted in the areas in which slow drying occurred.

The experiments showed that a severe anemia, high bile pigment concentration, and unusual drying of the blood are not the sole factors responsible for the creation of the phenomenon. The negative results suggested that the formation of ring bodies is dependent upon the presence of a circulating specific hemolysin which alters the relationship of the essential components of the protoplasm while in vivo, and that delayed drying, fixing, and staining of the blood are of lesser importance, but are contributory factors. That specific lysin may very likely be a bile derivative "bile acid" that disassociates the chemical components of certain erythroid cells not only in vivo but seemingly also in vitro is suggested by analytical data presented by Irvin, Johnston, and Sharp* on bile acids and their hemolyzing effect on the red blood cells in vitro.

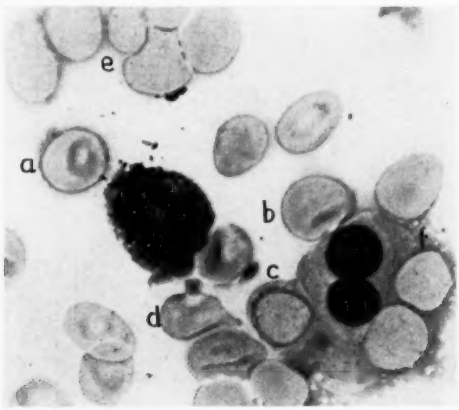

Fig. 3.—Mr. A. K. Peripheral blood showing the development of a Cabot ring body. *a*, Markedly lysed erythrocyte with a reddish corona. Phase I. *b*, Erythrocyte with a pale peripheral area which follows the contour of the cell (for its replica see Plate I, *1*). This phenomenon is produced by the retraction of the cytoplasm exposing the transparent envelope. Phase II. *c*, Erythrocyte with retracted peripheral ring. The cell was markedly lysed. The structure had the characteristic red color of Cabot ring bodies. Phase III. *d*, Poikilocyte with irregular aggregated protein at the periphery. *e*, Group of erythrocytes showing lysis corona suggesting peripheral rings. See Fig. 27 in Cabot's article. *f*, Erythroblast with nuclei approximating the diameter of the ring cell *c*, showing that Cabot's assumption that the bodies may represent nuclear remnants is feasible.

STRUCTURE AND TINCTORIAL BEHAVIOR OF THE CABOT RING BODIES

Experiments were designed to determine the reason for the fluctuation of the dimensions, configurations, and affinity to specific cytoplasmic dyes of the ring bodies.

Preparations were used which were made by the slanting method, showing from 12 to 34 erythrocytes with ring bodies per 180 micrometer squares. The location of all cells chosen for this critical study was determined with the vernier scale of a mechanical stage.

Photographs before and after decolorization revealed that some of the ring bodies were at or near the surface, and seemingly part of the envelope, whereas other rings seemed to belong to a layer or layers distinctly below the erythrocyte

*Annual meeting of the Central Society for Clinical Research, Chicago, Ill., Nov. 1, 1940.

SIMPSON MEMORIAL INSTITUTE
ANN ARBOR, MICH.

envelope. With the May-Grünwald Giemsa stain these ring bodies were either of a dubious reddish blue, or distinctly red or blue. The ring bodies assumed to be at or near the surface could be made out only with difficulty when decolorization was carried to a point where the nucleus of the leucocytes could still be made out. The deeper lying rings either retained their hue, were a nuance lighter, or became more distinctly red or blue. Some rings which showed irregular staining affinities were invariable composed of irregular thick bars (threads) or of tiny globules. Other ring bodies were composed of not only one, but of two or more thin rings either superimposed on each other or concentrically arranged.

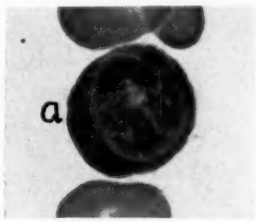

Fig. 4.—Mr. O. H. Pernicious anemia in severe relapse; peripheral blood. *a*, Basophilic megalocyte with a colorless protein ring. The ring, which had a reddish blue tint, could not be restained after the preparation was exposed to room temperature for one week.

Thus it may be assumed that the staining variability depends upon the character and quantity of the substance that makes up a ring, the molecular thickness of such a structure, and the momentary physical state of ring constituents. The latter may supply the answer to the loss of affinity for a dye of a ring body that formerly stained reddish blue. This phenomenon occurred in several basophilic megalocytes from a patient with pernicious anemia in severe relapse when a preparation was decolorized and restained after exposure to room temperature for six days. Fig. 4 shows an example of a colorless ring body in a basophilic megalocyte.

STRUCTURE OF THE MATURE ERYTHROCYTE

It is not within the scope of this article to give an account of the numerous hypotheses that have been advanced concerning the structural make-up of the mature erythrocyte. The literature on this particular subject is more confusing than enlightening. The contributions of Michels,[29] Ponder,[30] and others[6, 17, 31-34] give a general picture of this topic.

I have long favored the trend initiated by Ponder to divide the physicochemical structure of the erythrocyte into three components: (a) membrane, i.e., envelope, (b) cytoplasm, and (c) their respective protein-lipoid constituents. This division is not only unreservedly subscribed to by most investigators, but it permits projection of the laboratory experiments presented in this paper into the erythrocytes. All accumulated physicochemical data represent at best only relative values, since the constituents vary with the physiologic state of the subject. The protein component which, as shown elsewhere in this paper, is responsible for the formation of a ring body, constitutes less than 8 per cent of the cell.

Jorpes[35] refers to the protein proper as stroma protein and suggests that it has a characteristic composition different from other proteins of the blood. Its chief properties are (a) consistency, (b) elasticity, and (c) contractility.

Bechhold,[36] while studying the colloidal structure of the erythrocyte, observed the formation of "globules" in normal mammalian red blood cells during the process known as hemolysis. Salén,[37, 38] following up Bechhold's work, used human erythrocytes, and noted with the aid of an ultramicroscope the formation of a ring structure within the cell while it was subjected to a hypotonic saline solution and an acid. The ring was formed at or near the periphery of the cell, and as hemolysis progressed, the ring began to retract, finally degenerating into a refractile granule which eventually disappeared.

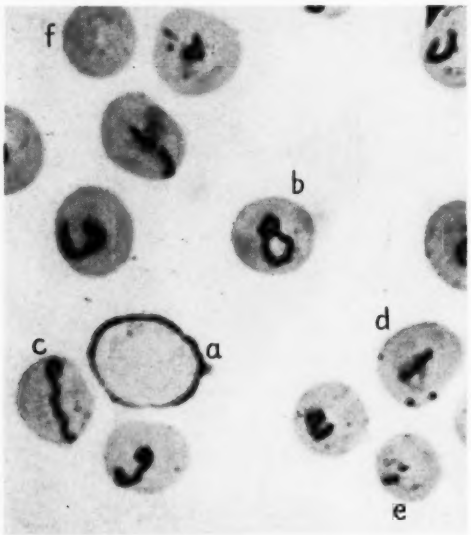

Fig. 5.—Normal human erythrocytes treated with Salén's method. *a*, Erythrocyte showing peripheral ring. *b*, Erythrocyte with two rings that originated at different planes forming a pseudofigure eight. *c*, Erythrocyte with disintegrating ring. Note the hook formation. *d*, Erythrocyte with bar and two globules. *e*, Erythrocyte with globules. *f*, Lysed erythrocyte (ghost cell).

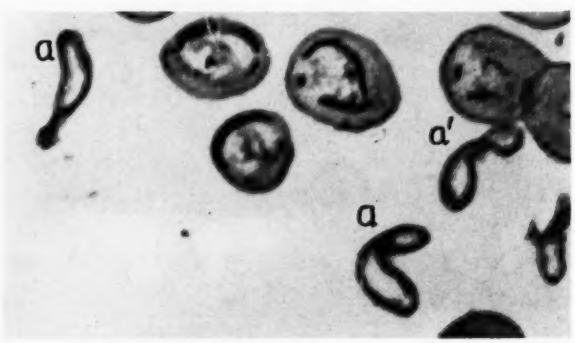

Fig. 6.—Normal human blood treated with Salén's method. *a*, Free protein rings. *a'*, Ring twisted into a figure eight.

Salén's observations were confirmed. Fig. 5 shows an erythrocyte with a peripheral ring, others with stab bodies, some with granules, and one cell without structures. Fig. 6 shows free rings obtained by mechanically rupturing erythrocytes.

Salén reached the following conclusion: "the ring structure seemingly consists of water-insoluble proteins which are precipitated by potassium ferrocyanide dissolved in dilute acetic acid." He thus furnished tangible evidence that a ring structure is formed when hemolysis sets in, thus associating the phenomenon with degeneration of protein.

After correlating the dye analytical studies with Salén's observations, it could be assumed that Cabot ring bodies might be identical with Salén's ring structures, especially since these bodies showed a selective affinity for cytoplasmic stains.

Thus, aggregated and denatured protein forms the Cabot ring bodies.

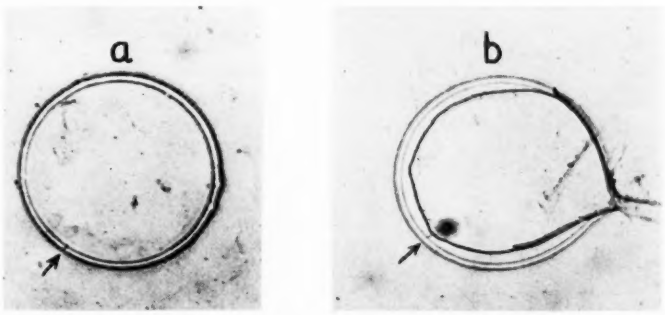

Fig. 7.—Protein globules of beaten egg white. *a*, Globule with two rings. The peripheral ring had a reddish blue color, the inner ring was tinted blue. The cross bar stained light red. See arrow. *b*, Ruptured globule. The broken top ring, which is partially extruded, had a deep blue color and was seemingly superimposed on another ring, also broken, that was reddish blue in hue. The two other rings and the cross bar (at arrow) had the same tint as the latter ring.

THE NATURE OF CABOT RING BODIES

The following experiments prove that the ring bodies are protein derivatives of the envelope and cytoplasm; and that the variability of affinity for acid and basic dyes is determined by the molecular thickness and momentary physicochemical state of the protein mass.

Experiments With Egg White.—Experiment 1. The white of a raw egg was carefully separated from the yolk and beaten (or shaken in a test tube) until it was stiff. With the aid of a wire loop a small amount of the substance was transferred to a clean glass slide and spread to a thin film with a suitable spreader, cover slip, etc. The film was permitted to dry, then covered with Wright's blood stain for one minute, after which the stain was diluted with an equal amount of distilled water. The diluted stain was allowed to act for two to three minutes. The preparation was then rinsed in distilled water and permitted to air dry. Such preparations not only showed numerous single globules, but the globules varied also in size and thickness, and thus in some respects resembled normal and pathologic protein quantities of an erythrocyte, i.e., the globules are analogous to the erythrocyte envelope. The majority of the globules showed one or two, and less frequently three or more, reddish-blue rings, either concentrically arranged, or superimposed upon each other. Fig. 7 shows two representatives of the globules; the globule to the right, ruptured either during the drying process or during the staining procedure, shows a striking difference in thickness of the rings; a replica of Cabot rings in every respect.

Experiment 2. A thin film of unbeaten egg white was made on a microslide, and the substance was exposed to room temperature for at least one hour and then stained with Wright's stain in the usual way. In such a preparation there are areas in which many various-sized round to oval structures may be seen that show generally one concentric complete ring, and variable types of incomplete structures. A typical example is shown in Fig. 8, a replica in every respect of Fig. 1. When two thin films are superimposed upon each other, the formation of a figure eight is frequently encountered. Fig. 9 shows the manner in which such a configuration is produced. These protein structures are broader and flatter than those produced with beaten egg white.

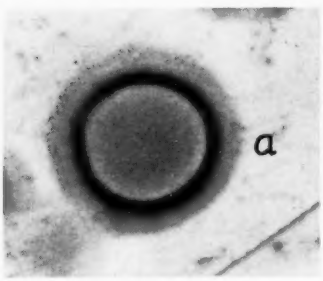

Fig. 8.

Fig. 9.

Fig. 8.—Protein ring bodies of unbeaten egg white. *a*, Spherical body with deep blue flat ring. Note the uniformity of staining of the outer and inner area of the mass. A striking simulacrum of Fig. 1*a*.

Fig. 9.—Protein configurations of two thin films of unbeaten egg white. Incomplete figure eight. Note that two spherical bodies at different planes are involved in its formation.

Thus variability of dimensions, i.e., thickness of Cabot ring bodies, depends upon the availability of protein within the envelope or cytoplasm of a given erythrocyte.

The experiments showed that protein, regardless of its source, forms predominantly circular structures. The following hypothesis is offered for the presence of more than one ring body in an erythrocyte:

It is assumed that the human red blood cell envelope is not homogeneous, but consists of a series of concentric lipoproteid layers which under certain conditions may separate, in contrast to the cytoplasm which is a homogeneous mass, and thus merely retracts, behaving the same way as a thin layer of unbeaten egg white. Thus it is not at all difficult to account for the occurrence of more than one Cabot ring body at the same or at different planes within an erythrocyte, as well as for the thickness and configuration of the protein structures, as shown in Plate I.

DIFFUSE AND PUNCTATE BASOPHILIA

It is a well-known fact that Cabot ring bodies may appear in an erythrocyte showing either diffuse or punctate basophilia. Pappenheim[39] believed that polychromatophilia, punctate basophilia, and reticulation represent diffuse or aggre-

gate states of the spongioplasm. Many investigators, among them Key, Daum, and Cooke, have shown that Pappenheim's hypothesis was based on sound observations. Key[40] proved by appropriate methods that a basic substance retained by certain erythrocytes may be made to appear either as polychromatophilia, reticulation, or punctate basophilia, the latter representing an aggregation of the basophilic substance in the cell due to a pathologic process. Daum[41] showed that punctate basophilia may be created in vitro by permitting normal defibrinated blood to stand at room temperature for twenty-four to thirty-six hours. She observed that as autolysis progressed, diffused polychromatophilia changed to stippling, and as time went on, the punctate basophilia became more and more coarse, apparently in direct proportion to the autolysis. Daum came to the conclusion that the basophilic substance is most likely a colloid that is denatured and coagulated by heat, alcohol, or any other agent. Pappenheim thought the substance to be a lipoid, as opposed to others who assumed the basic substance to be albuminous. Cooke,[42] using the blood smear method, employed a benzidine-alcohol-hydrogen peroxide mixture for the demonstration of punctate basophilia and reticulation in normal mature erythrocytes. He reached the following conclusion: "When obtained by the usual staining methods, this would point to increased permeability or a defect in the lipoid envelope of the erythrocyte. There may be, and probably is, a close relationship between punctate basophilia, diffuse polychromatophilia, diffuse polychromasia, and reticulation and maturity." Cooke's conclusion supports the observation of Falconer,[43] of the occurrence of basophilic stippling in mature erythrocytes in the blood of normal healthy persons. On analysis of the evidence derived from the literature, it may be assumed that basophilia, polychromatophilia, reticulation, and punctate basophilia (stippling) are nothing more than the morphologic expression of denatured and coagulated colloid substances, i.e., **lipoproteid colloids.**

THE PRODUCTION OF DIFFUSE AND PUNCTATE BASOPHILIA WITH EGG YOLK

The experiments with egg white did not produce the phenomenon known as diffuse and punctate basophilia, and thus Pappenheim's assumption that lipoids may be responsible, was apparently supported. The following experiments were designed to obtain information concerning the role played by lipoids.

Experiment I. A thin layer of unbeaten egg yolk was made on a microslide, air dried, and stained with Wright's blood stain. The preparation revealed a homogeneous layer of a more or less reddish-purple hue.

Experiment II. A thin film of unbeaten egg white was made on a microslide and permitted to dry at room temperature. A thin layer of unbeaten egg yolk was superimposed and permitted to dry. The preparation was tinted with Wright's blood stain, air dried, and mounted in Clarite. Numerous round and oval bodies were seen that showed the well-known tinctorial characteristics of diffuse and punctate basophilia. The experiment showed that the presence of a protein layer enhances aggregation of lipoids. Fig. 10 shows several of these bodies. Thus the following opinion may be expressed: 1. The various tinctorial shades of diffuse basophilia are apparently the expression of a more or less

homogeneous state of protein and lipoids. 2. Punctate basophilia may be interpreted as the result of disassociation of these substances, or the independent reaction of the laminated lipoid component.

Thus it is understandable why a ring body can appear in an erythrocyte exhibiting either diffuse or punctate basophilia. Fig. 11 shows a polychromatic red blood cell with a faintly stained ring body and punctate basophilia superimposed.

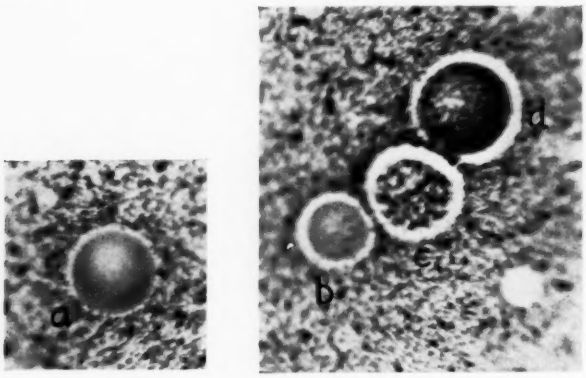

Fig. 10.

Fig. 11.

Fig. 10.—Egg white and egg yolk superimposed upon each other. *a*, Bluish round body resembling a basophilic erythrocyte. *b*, Round body (slightly out of focus) with fine granules which stained reddish blue. *c*, Round body with coarse blue granules. A striking replica of punctate basophila. *d*, Round body with coarse deep blue and reddish blue granules. The white zone around the bodies is the result of retraction of the egg white.

Fig. 11.—Mr. R. P. Acute lead poisoning; peripheral blood. *a*, Orthochromatic erythrocyte with a light red protein ring and superimposed dark blue globules.

EXPERIMENTS WITH NORMAL HUMAN ERYTHROCYTES

As previously pointed out, the prerequisite for the formation of Cabot ring bodies is a physicochemical imbalance of the protein-lipoid components of the cell, permitting disintegrating agents to permeate and to precipitate the stroma protein. While these constituents are properly balanced, they form a definite structural unity. The process which supposedly modifies the envelope of the red blood cell is called "hemolysis," assumed to be a reaction between a lysin and the protein. It is obvious that the production of a simulacrum of the Cabot ring bodies in normal erythrocytes is possible only if the erythrocyte is exposed to a hemolysin that modifies the permeability of the envelope. If colloidal proteid is the substance that through denaturation forms a ring structure, then the problem at hand is to set up experiments that first assured hemolytic action, i.e., conditioning of the envelope in such a manner that denaturation of the protein is assured, followed by the penetration of a dye which would stain the protein.

The oldest and most effective means of altering envelope permeability is heat. This thermal factor, while a weak hemolysin, is responsible for many artifacts in cytology, but under controlled conditions is a valuable agent, especially when combined with strong alcohols and weak acids. The investigations carried out by Foà[44] on mammalian and human blood, and by Dehler[45] on domestic fowl, brought out the fact that the entrance of dissolved substances, such as dyes, into

the envelope and cytoplasm, is more rapid at high temperatures than at low temperatures. This knowledge has been utilized in devising an experiment for the creation and staining of replicas of "synthetic" Cabot ring bodies. While studying the variability of the envelope resistance, I consulted the papers by Weidenreich[46] and Sakai.[47] The latter, as already pointed out, produced ring bodies, but failed to suggest their similarity to Cabot rings. The striking replicas obtained by Sakai made his method the one of choice for preliminary work. The method, however, had to be modified for the use of human erythrocytes. Below is Sakai's method as I modified it:

I. *Solutions*

1. Ethyl alcohol, absolute.
2. Phenol (crystals C.P.) 2 per cent aqueous solution.
3. Phenol-gentian violet solution. (Add 5 Gm. of gentian violet to 100 c.c. of pure acetone-free methyl alcohol.)
   Take 40 c.c. of solution (2) and 10 c.c. of solution (3), mix well, and let stand at room temperature for 24 hours. Filter and transfer to a staining jar. The dye is good for only 24 to 36 hours.

II. *Method*

1. Make a thin blood smear and let air-dry for 5 hours.
2. Place into ethyl alcohol for 5 minutes.
3. Place into phenol solution 2 per cent for 30 seconds.
4. Place into phenol-gentian violet for 2 minutes.
5. Rinse in distilled water for 15 seconds.
6. If counterstain is desired, cover preparation with diluted Giemsa solution for 5 minutes.
7. Rinse in distilled water and let air-dry.
8. Mount with dammar or Clarite.

In a blood smear subjected to the foregoing treatment, the erythrocytes have a bluish tint, and their contour is sharply defined and not distorted. At the periphery a narrow, somewhat lighter area follows the shape of the cell. This phenomenon is apparently a sequela of the formation of a ring body; it can be seen in Fig. 3, cell *b*. A well-defined flat and dark-stained blue ring structure, at some distance from the periphery, is present in every mature erythrocyte, a striking replica of a Cabot ring body (Plate I, *1*). The ring structure seems to be derived from a layer or layers at or near the surface, or adjacent to the envelope. Frequently two ring structures may be seen. Cell degeneration is thus seemingly expressed as complete or incomplete rings, or a ring is suggested by a ringlike arrangement of beads or rods. Cell immaturity can be recognized by the decreasing definition of the ring structure, which eventually is replaced by more or less fine granules which may fuse and form coarse clumps. The percentage of the latter types corresponds to that of the reticulocytes, Fig. 12, cells *c, e, f*. The amount of basophilic spongioplasm within the cell apparently determines the respective pattern. These standard patterns are thus the expression of the physicochemical status of an erythrocyte. In normal basophilic erythroblasts the spongioplasm is diffuse and deeply staining. Fig. 12 shows a characteristic pattern obtained with the method. Fig. 13 shows an erythroblast in mitosis, late in telephase stage. The lipoproteid colloids may well be in a transition stage, i.e., the respective quantities are as yet not properly assimilated. If this is the case, then the question of why Cabot ring bodies are so rarely seen

in erythroblasts is answered. However, bizarre patterns may be observed in severe anemic bloods, especially when cytolysis of erythroblasts is pronounced. When such blood is exposed to the phenol-gentian violet solution, the erythroblasts may occasionally show incomplete rings, but generally, fine or coarse granules, rods, spotted or diffuse spongioplasm, or combinations of the enumerated patterns may be seen. When in a routinely stained blood smear an erythroblast with a Cabot ring body is observed, the cell is most likely abnormal, as shown in Fig. 14.

The experiment revealed that replicas of Cabot ring bodies may be produced in normal mature erythrocytes but not in normal erythroblasts. The creation of the bodies depends upon the physicochemical state of the lipoproteid colloids at the various maturation stages. Further, an explanation is furnished for the rare occurrence of Cabot ring bodies in erythroblasts. There is no doubt in my mind of the identity of the simulacrums created by the afore-mentioned method and the Cabot ring bodies. Both structures are the expression of denatured protein.

GLACIAL ACETIC ACID METHOD FOR THE CREATION OF DENATURED PROTEIN CONFIGURATIONS (CABOT RING BODIES) IN NORMAL MATURE ERYTHROCYTES

This method was based upon the well-known facts that weak acids and strong alcohols are hemolysins which in turn denature protein. The method has the advantage of reducing the room temperature exposure time. Heating the stain was found to facilitate rapid penetration.

I. *Solutions*

1. Wright's blood stain.
2. Glacial acetic acid 0.02 per cent.
3. Clarite or dammar.

II. *Method*

1. Permit the blood smear to air-dry at room temperature for one hour.
2. Place 20 drops of Wright's blood stain in a test tube, and heat over a flame to 50° C. Pour the dye quickly over the blood film, starting at the thick end.
3. Dilute the dye with 60 drops of glacial acetic acid 0.02 per cent. Mix well with an eye dropper by drawing in and expelling the dye.
4. Direct a flame under the glass slide and heat the dye solution to 50° C. This temperature is generally reached when the solution begins to steam.
5. Permit the hot dye to react for one minute. Rinse in distilled water for a few seconds and then let the preparation air-dry.
6. Mount with dammar or Clarite.

With the method described above patterns resulted identical to those created with the phenol-gentian procedure. When, however, the stain and acetic acid are heated together and permitted to act about one minute, many interesting patterns are obtained. Fig. 15 shows an example pattern that is produced with this modified procedure when blood samples of severe toxic and chronic anemias are used. The incomplete removal of the hemoglobin is responsible for the bizarre patterns. There is no erythroblast in the field.

DISCUSSION

While Cabot suggested that the ring bodies he described may represent nuclear remnants, it seemingly remained for Ferrata, Viglioli, and others to show

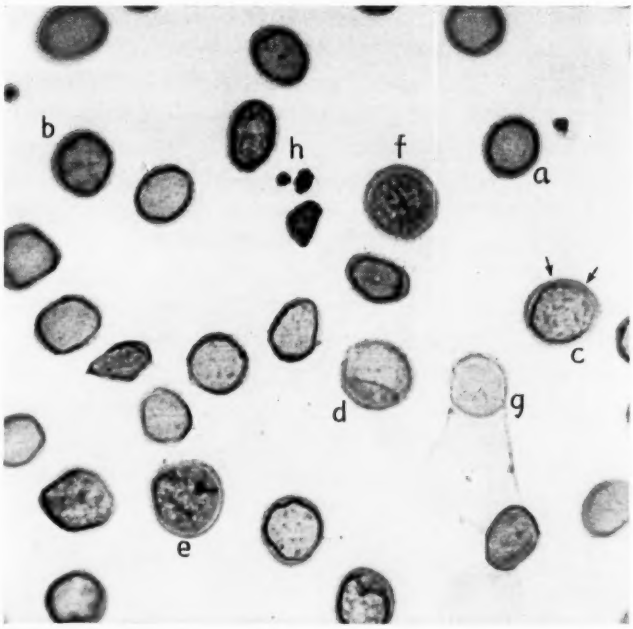

Fig. 12.—Mrs. B. W. Pernicious anemia in severe relapse. Peripheral blood treated with the author's acetic acid method. *a*, Normal mature erythrocyte with a characteristic protein ring. Note the striking resemblance to Fig. 1*a* and Fig. 8*a*. *b*, Mature erythrocyte with a ring body and a globule. *c*, Macro-erythrocyte with two ring bodies. The peripheral ring was light red, the other one was partially red and blue. Note the cross bars connecting the rings. A replica of the bars is seen in Fig. 7*a* and *b*. *d*, Macro-erythrocyte with incomplete light red rings and two blue globules. *e*, Megalocyte with incomplete ring, two globules, and a bar. The granular background is basophilic substance. *f*, Megalocyte (reticulocyte stage) more immature than cell *e*. The cytoplasm has retracted away from the periphery, leaving a light zone. *g*, Erythrocyte with the envelope ruptured. *h*, Thrombocytes.

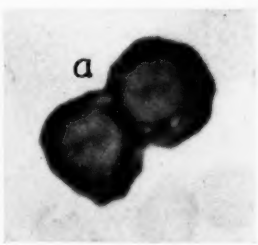

Fig. 13.

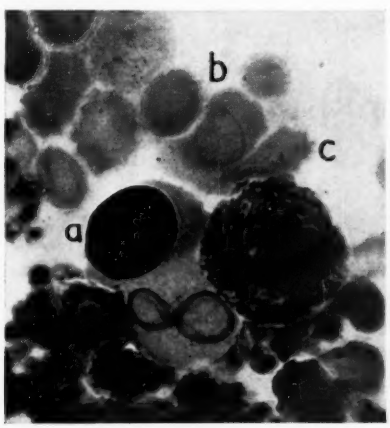

Fig. 14.

Fig. 13.—Mr. F. M. Normal bone marrow treated with the acetic acid method. *a*, Erythroblast, late telophase. Note the basophilic spongioplasm, sharply defined nuclear membrane, and blotches in the cytoplasm. The constituents of the envelope and cytoplasm are seemingly not properly assimilated, and this may account for the absence of rings in erythroblasts. In the few exceptions cited, it is assumed that protoplasmic maturation was further advanced and that this led to the production of ring bodies.

Fig. 14.—Mr. A. K. Generalized Hodgkin's disease; peripheral blood. *a*, Pathologic polychromatic erythroblast with two twisted protein rings. The darker structure had a deep red color; the lighter one, lying at a lower plane, had a blue hue. *b*, Markedly lysed polychromatic erythrocyte with a red protein ring. *c*, Ruptured erythrocyte with the cytoplasm flowing around cell *b*. The ring had a reddish blue tint and appeared to belong to the envelope.

that the structures are identical with the nuclear membrane. This prevailing view, however, is based on misinterpretation of artifacts so commonly encountered in cytology. The literature dealing with the subject is extensive, and contains many diametrically opposed theories regarding the origin and nature of the Cabot ring bodies. None of the investigators has furnished convincing proof that the structures are either nuclear derivatives or artifacts, and thus laboratory creations. I have attempted to show that the Cabot ring bodies are actually the expression of denatured protein, i.e., colloid protein, and thus that the bodies have no immediate relation to the nuclear membrane. The ring bodies created at will in the laboratory, by slanting the glass slide while making the blood smear, and thereby delaying the drying process in a certain area, are in every respect identical with the genuine structures.

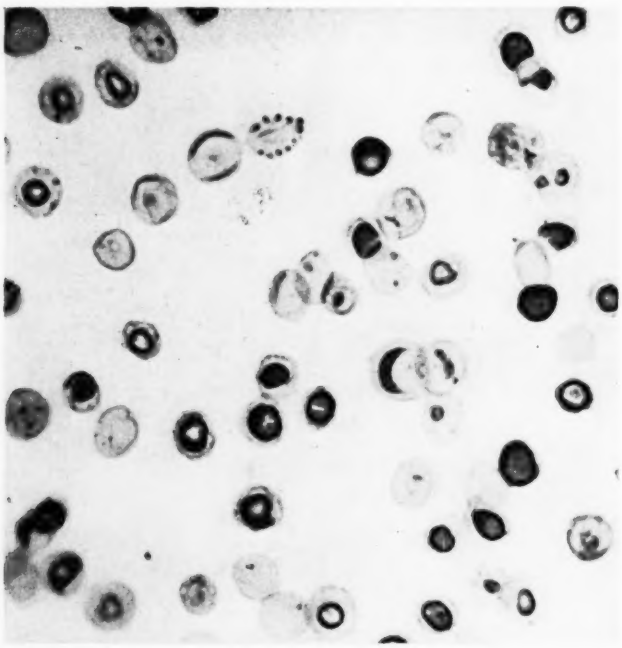

Fig. 15.—Mr. G. E. Chronic toxic anemia (carcinoma of the cecum), treated with the acetic acid method (modified). The variability of the erythrocyte envelope to lytic agents is well demonstrated. In some cells which have been partially hemolyzed the hemoglobin simulates nuclear dissolution (Ferrata). Other cells conform to Demel's theory. There is no erythroblast in the field.

The replicas were produced in the anemic blood of patients with pernicious anemia, acute lead poisoning, terminal lymphosarcoma, acute malaria, terminal lymphatic leucemia, congenital hemolytic icterus during hemolytic crisis, erythroblastosis fetalis, and terminal malignant Hodgkin's disease. According to the literature, these toxic and hemolytic anemias furnished, for the most part, the material for Cabot ring bodies in routine blood smears, and thus there can be little doubt that hemolysis plays an important role as a preconditioning factor for the production of the structures.

It has been shown that Cabot ring bodies can occur only in cells in which the permeability of the envelope has been altered by a specific lysin. The reason for the preponderance of flat circular bodies lies in the assumed geometry

of the erythrocyte and the tendency of protein to aggregate into a ring structure. The rarity of Cabot ring bodies in erythroblasts is assumed to be due to either a peculiar quantitative or a qualitative state of the lipoproteid colloids, or these constituents at this particular developmental stage have not yet assumed their mature relationships. An explanation has been offered for the presence of both a ring body and punctate basophilia in the same erythrocyte by showing that ring structures are aggregations of protein and punctate basophilia is identified with lipoids. Simulacrums of both phenomena were reproduced with egg white and egg yolk, and by special methods in normal mature erythrocytes.

The formation of a Cabot ring body is seemingly as follows: a specific hemolytic agent (bile acid) affects a certain erythrocyte whether basophilic, polychromatic, or orthochromatic; the lysin is injurious to the envelope, i.e., it produces changes either qualitative or quantitative, or both, in the lipoprotein constituents of the surface layer which not only influences the permeability but also may lead to microscopic tears in the envelope. This physicochemical change encourages disassociation of the lipoproteid layers, leading to a separation of the normally fused layers into individual ones, or to the separation of a mass of fused layers. This irregular splitting produces the variability of flatness and thickness of the ring bodies. This segregation of protein layers occurs during the drying process, leading either to the formation of the protein configurations known as Cabot ring bodies, or to such structures as globules or bars, as shown in Plate I, *5* and *10*. The answer to Cabot's query "why," the reddish blue, red, or blue color of the ring bodies lies in the very nature of the structures.

CONCLUSION

The origin and nature of the Cabot ring bodies of erythrocytes have been discussed and demonstrated by special methods. Cabot ring bodies are neither nuclear remnants nor are they identical with the nuclear membrane; they are laboratory creations, the expression of cellular degeneration induced by hemolytic agents, i.e., they are the expression of aggregated and denatured colloid protein.

I wish to thank Dr. Hal Downey, who offered numerous helpful suggestions, and who was kind enough to edit the manuscript; Dr. R. A. Gortner, Head of the Department of Agricultural Biochemistry, University of Minnesota, for his aid in connection with the chemical aspect of the problem; F. N. Ruslander, medical photographer, Detroit, Mich., for the care exercised in preparing the photomicrographs. I am also grateful for the personal communications of the late Dr. R. H. Jaffé, whose genuine interest in the problem stimulated the investigation.

REFERENCES

1. Cabot, R. C.: Ring Bodies (Nuclear Remnants?) in Anemic Blood, J. M. Research **9**: 15, 1903.
2. Schleip, K.: Über Ringkörper im Blute Anämischer, Deutsches Arch. f. klin. Med. **91**: 449, 1907.
3. Schleip, K., and Alder, A.: Atlas der Blutkrankheiten, ed. 3, Berlin, 1936, Urban & Schwarzenberg.
4. Pappenheim, A.: Über Polychromophilie, Folia haemat. **9**: 318, 1910.
5. —: Folia haemat. Arch. **9**: 304, 1910.
6. —: Folia haemat. Supplement, pp. 48 and 302, 1907.
7. Sluka, E.: Schleifenbildung in polychromatischen und basophil gekörnten roten Blutkörperchen, Deutsches Arch. f. klin. Med. **93**: 577, 1908.
8. Alder, A.: Atlas des normalen und pathologischen Knochenmarkes, Berlin, 1939, Urban & Schwarzenberg.
9. Ferrata, A.: Über die klinische und morphologische Bedeutung der vitalfärbbaren Substanz und die basophilic Punktierung der Erythrocyten, Folia haemat. **9**: 268, 1910.
10. —: Le Emopatie, **1**: 37, 1933. Soc. Editrice Libraria, ed. 2, Milano.

11. Ferrata, A., and Viglioli, G.: Über die Cabotschen Ringkörper, die azurophilen Granulationen und die azurophile Polychromatophilie der Erythrocyten, Folia haemat. Arch. **11**: 315, 1911.
12. Juspa, V.: Über den Entstehungsmechanismus der Cabotschen Körper und ihre diagnostische Bedeutung bei den experimentellen Anämien und den schweren Anämien des Menschen, Folia haemat. **17**: 429, 1914.
13. Jordan, H. E., Kindred, J. E., and Beams, H. W.: The Relation of Cabot Rings to the Reticulofibrillar Apparatus of Golgi, Anat. Rec. **46**: 139, 1930.
14. Demel, A. C.: Sulle formazioni endoglobulari pseudonucleari e sugli anelli de "Cabot" messi in relievo nei globuli rossi normali colla ipercolorazione, Haematologica **2**: 125, 1921.
15. Russow, K. E.: Über Ringkörper in Blute Anämischer, Deutsches Arch. f. klin. Med. **102**: 210, 1911.
16. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, ed. 5, Berlin, 1931, Julius Springer.
17. Schilling, V.: Arbeiten über den Erythrocyten, Folia haemat. **14**: 95, 1912.
18. —: Das Blutbild und seine klinische Verwertung, ed. 6, Jena, 1926, Gustav Fischer.
19. Schröder, E.: Über die Erythrokonten, Folia haemat. **48**: 1, 1932.
20. Isaacs, R.: The Erythrocyte in Downey's *Handbook of Hematology*, New York, 1938, Paul B. Hoeber, Inc., Vol. I, page 25.
21. Isamboulas, N., and Malikiosis, X.: Zur Frage der Cabotschen Ringkörper, Deutsches Arch. f. klin. Med. **184**: 183, 1939.
22. Schleicher, E. M., and Sharp, E. A.: Rapid Methods for Preparing and Staining Bone Marrow, J. Lab. & Clin. Med. **22**: 949, 1937.
23. Limarzi, L. R., and Schleicher, E. M.: The Reaction of Peripheral Blood and Bone Marrow in Chronic Hemorrhage and in Essential Thrombocytopenic Purpura, J. A. M. A. **114**: 12, 1940.
24. Sharp, E. A., and Schleicher, E. M.: Hemologic Observation on Sickle Cell Anemia, Am. J. Clin. Path. **6**: 580, 1936.
25. Schleicher, E. M.: Schilling's Hemogram, J. Lab. & Clin. Med. **6**: 1296, 1936.
26. —: Estimation of Erythrocytes From a Blood Smear by a "Dry Chamber" Method, Proc. Soc. Exper. Biol. & Med. **40**: 536, 1939.
27. Lepeschinskaja, O. B.: Zur Frage der Erythrocytenmembrane, Folia haemat. **31**: 87, 1924.
28. —: Die Erythrocytenmembran als kolloides System und ihre Veränderungen, Folia haemat. **36**: 41, 1928.
29. Michels, N. A.: The Erythrocyte, Haematologica, II, Recensioni **2**: 101, 1931.
30. Ponder, E.: The Mammalian Red Cell and the Properties of Haemolytic Systems, Berlin, 1934, G. Borntraeger.
31. Schilling, V.: Erythrokonten Stäbchen in Erythrocyten, bei Anämia Perniciosa und einigen nahestehenden Krankheitsbildern, Klin. Wchnschr. **7**: 785, 1928.
32. Rinehart, J. F.: Intracellular Precipitation and Impregnation of Hemoglobin in the Erythrocyte, Folia haemat. **48**: 231, 1932.
33. Kasahara, M., and Nagahame, M.: Über das Auftreten von silberimprägnierten Erythrocyten bei infantiler Bleianämie, Folia haemat. **56**: 221, 1936.
34. Lepeschkin, W. W.: Über das Wesen der Hämolyse, Folia haemat. **54**: 53, 1935.
35. Jorpes, E.: The Protein Component of the Erythrocyte Membrane or Stroma, Biochem. J. **26**: 1488, 1932.
36. Bechhold, H.: Bau der roten Blutkörperchen und Hämolyse, München. med. Wchnschr. **68**: 127, 1921.
37. Salén, E.: Kolloidstudien über den Bau der roten Blutkörperchen und ihre Hämolyse, Biochem. Ztschr. **110**: 176, 1920.
38. —: Die roten Blutkörperchen in Dunkelfeldbeleuchtung, München. med. Wchnschr. **68**: 885, 1921.
39. Pappenheim, A.: Neue Zytomorphologische Studien an Blutzellen mit farbenanalytischen Methoden, Folia haemat. **9**: 572, 1910.
40. Key, J. A.: Studies on Erythrocytes, With Special Reference to Reticulum Polychromatophilia and Mitochondria, Arch. Int. Med. **28**: 511, 1921.
41. Daum, H.: Über die Urform der basophilen Substanz in den Erythrocyten, Folia haemat. **53**: 1, 1934.
42. Cooke, W. E.: The Artificial Production of Punctate Basophilia and Reticulation in Erythrocytes, Am. J. M. Sc. **177**: 537, 1939.
43. Falconer, E. H.: The Clinical Significance of Punctate Basophilia in the Erythrocyte, Ann. Int. Med. **12**: 1429, 1939.
44. Foà, P.: Beitrag zum Studium der Structur der rothen Blutkörperchen der Säugetiere, Beitr. z. path. Anat. u. z. allg. Path. **5**: 255, 1889.
45. Dehler, A.: Beitrag zur Kenntnis des feineren Baues der roten Blutkörperchen des Hühnerembryo, Arch. f. mikr. Anat. **46**: 414, 1895.
46. Weidenreich, F.: Studien über das Blut und die blutbildenden und zerstörenden Organe, Arch. f. mikr. Anat. **61**: 459, 1903.
47. Sakai, K.: Über die Färbung der Erythrozytenmembran, Folia haemat. **46**: 401, 1932.

THE TREATMENT OF GONORRHEAL URETHRITIS IN THE MALE WITH SULFONAMIDE DERIVATIVES*

A Study of 199 Cases

LEROY W. LA TOWSKY, M.D., M.Sc., FRANK KNIGHT, M.D.,
CHARLES A. W. UHLE, M.D., AND RICHARD B. BAKER, M.D.
PHILADELPHIA, PA.

IT IS AN established fact that the use of sulfanilamide in gonococcic infections is not indicated since the later sulfonamides have been introduced. Pelouze[1] states that this substance was effective in only 25 to 40 per cent of over 900 cases treated by the Cooperating Clinics of the United States Public Health Service. Excluding sulfanilamide from the therapeutic armamentarium, there are three sulfonamide derivatives whose value has been established in the treatment of gonorrheal urethritis in the male. These substances are sulfapyridine, sulfathiazole, and sulfadiazine.

The authors have previously reported their experiences with these three substances in the treatment of gonorrhea in the male.[2-5] Though the number of cases we treated is not large, we believe that we are able to make some comparison of the therapeutic efficiency and action of these three sulfonamide derivatives. The basis for this report is the study of 199 patients with gonorrhea, who presented themselves for treatment, at the Genito-urinary Clinic of the Philadelphia General Hospital during the years 1939 to 1941.

Of the 199 cases of gonorrheal urethritis studied in the male, 167 were followed to the final step of either apparent cure or failure, while 32 patients defaulted. The average age of the 199 patients was 25 years, but their ages ranged from 11 to 54 years. One hundred and seventy-two of the patients were black, 26 were white, and one was Chinese. One hundred and forty-three had no treatment of any kind before coming to our clinic, while 56 had some type of treatment. Twenty-six had purchased sulfanilamide from druggists. One hundred previously had a gonorrheal infection, while 99 never had gonorrhea.

The classification of gonorrhea in the male, which was used in this study, is that of Eisendrath and Rolnick. They classify gonorrheal infection as follows[6]:

Acute urethritis (up to 3 months)
- Anterior
- Posterior

Subacute urethritis (3 to 6 months)
- Anterior
- Posterior

Chronic urethritis (6 months or more)
- Anterior
- Posterior

*From the Genito-urinary Clinic, the Philadelphia General Hospital, Philadelphia.
Received for publication, May 24, 1941.

Of the 199 patients who presented themselves to our clinic for treatment, there were 130 with acute anterior urethritis, 59 with acute posterior urethritis, 3 with subacute anterior urethritis, 6 with subacute posterior urethritis, and one with chronic posterior urethritis. Of the 156 patients who were eventually cured by these sulfonamide derivatives, 18 had epididymitis, 4 had gonorrheal arthritis, 4 had balanoposthitis, one had a periurethral abscess, and one had inguinal adenitis before therapy was begun.

The diagnosis of gonorrheal urethritis was based on the history, clinical symptoms and signs, and positive bacteriologic studies. These bacteriologic studies consisted of a smear and culture of the urethral exudate in every case. All stains were done by the Gram technique. The cultural technique used to identify the gonococcus was as follows*:

The urethral exudate or prostatic fluid is collected on a cotton swab. This swab is previously moistened with sterile physiologic saline. The swab is streaked on chocolate blood agar plates,† which are then incubated‡ at a temperature of 37.5° C. for twenty-four to forty-eight hours. At the end of this period typical colonies are isolated and placed on chocolate blood agar slants. These slants are incubated at the same temperature for twenty-four to forty-eight hours. The culture on the slant is tested for purity by the Gram stain technique, and sugar tubes, containing dextrose, maltose, and levulose with a broth base, are incubated at the same temperature for twenty-four to forty-eight hours at 37.5° C. Gonococci ferment only dextrose with the production of acid and no gas; they do not ferment the other sugars. From the chocolate blood agar slants the organisms are also tested by the Alkali Solubility Test.§

The patients were seen twice a week during the early stages of treatment, and later at intervals of one or two weeks. At each visit the customary urologic examinations were made, including bacteriologic work at appropriate time intervals. Complete studies of the cellular elements of the blood were made in 55 cases before and after treatment with the drugs. Electrocardiographic tracings were taken before and after treatment in 51 cases. Blood levels of the sulfonamide were made on almost every patient. The technique of Bratton and Marshall was used in these analyses.[7]

In the beginning of the study it was decided to keep the dosage as uniform as possible, yet to individualize the dose to fit the patient's disease. The drugs were continued for a few days after the patient was free of discharge or the urine had become clear in both glasses.

All cases received the drug in divided doses, usually two, three, or four times a day. With sulfapyridine the average dose to effect a cure was 23.5 Gm. given

*This technical aspect of the study was done by Drs. H. A. Shelanski and L. Shelanski, of the Department of Zoology, the University of Pennsylvania.

†The composition of chocolate blood agar is as follows: Difco proteose No. 3 agar. Add 0.3 per cent whole defibrinated horse blood. Add blood when agar is 70° to 75° C. to get chocolate agar.

‡All cultures are placed in an atmosphere of 10 per cent carbon dioxide and an excess of moisture when they are incubated.

§The procedure of the alkali solubility test is as follows: 0.1 N sodium hydroxide is added to a suspension of bacteria. If the bacteria are gonococci, there will be complete lysis of the organisms. This does not occur if the organisms are meningococci or *N. catarrhalis*.

over a period of eight days. The range of dosage was 14 to 111 Gm. given over a period of from seven to thirty-seven days. With sulfathiazole the average dose to effect a cure was 28 Gm., given over a period of eight days. The range of dosage was 12 to 48 Gm., given over a period of four to twenty-eight days. With sulfadiazine the average dose to effect a cure was 17.5 Gm., given over a period of eight days. The range of dosage was from 4 to 58 Gm., given over a period of from four to twenty-one days (see Table I).

TABLE I

CHEMOTHERAPY IN GONORRHEAL URETHRITIS IN THE MALE

SUMMARY OF ESSENTIAL DATA

	SULFAPYRIDINE 57 CURED CASES	SULFATHIAZOLE 48 CURED CASES	SULFADIAZINE 51 CURED CASES
Dosage to effect a cure	Average: 23.5 Gm. in 8 days Range: 14 to 111 Gm. in 7 to 37 days	Average: 28 Gm. in 8 days Range: 12 to 48 Gm. in 4 to 28 days	Average: 17.5 Gm. in 8 days Range: 4 to 58 Gm. in 4 to 21 days
Duration of discharge	Average: 3 days Range: 2 to 8 days	Average: 3 days Range: 1 to 7 days	Average: 4 days Range: 1 to 30 days
Time elapsing from beginning treatment until appearance of first negative prostatic culture	Average: 51 days Range: 14 to 162 days	Average: 28 days Range: 7 to 88 days	Average: 13 days Range: 3 to 43 days
Time elapsing from cessation of symptoms until last positive culture of the prostatic fluid (the carrier state)	Average: 17 days Range: 2 to 50 days	Average: 17 days Range: 3 to 53 days	Average: 3 days Range: 0 to 18 days

The provocative tests were begun when clinical symptoms had ceased, and the urine had remained clear for three or four days. These tests were begun early because of the type of patients with which we were dealing. Most of the patients had a tendency to default when they began to feel that they were improving. The following consecutive tests were required of a patient before he could be discharged as cured: (1) Prostatic massage, smear and culture of fluid; (2) alcoholic indulgence; (3) passage of a sound into the urethra; (4) examination of a condom specimen; (5) repeated prostatic massages, both smear and culture of the prostatic fluid to be negative for gonococci on two or more occasions.

The results of treatment of gonorrheal urethritis in the male with the three sulfonamide derivatives are summarized in Table II. Of the 199 patients studied, 32 (16.2 per cent) defaulted and 167 (83.8 per cent) were followed to the completion of the cure, or failure. Of the 167 patients followed, 156 (93.5 per cent) were apparently cured, as evidenced by satisfying all criteria of cure, and 11 cases (6.5 per cent) were failures. Most of the patients treated had anterior urethritis, but all forms of acute and subacute urethritis were represented in this study.

Of 62 cases treated with sulfapyridine and followed, 92 per cent were apparently cured. Of 50 cases treated with sulfathiazole and followed, 96 per cent

were apparently cured, and of 55 cases treated with sulfadiazine and followed, 93 per cent were apparently cured. There is no significant statistical difference between these cure rates.

TABLE II

TREATMENT WITH SULFONAMIDE DERIVATIVES OF GONORRHEAL URETHRITIS IN THE MALE
AN ANALYSIS OF 199 CASES

Philadelphia General Hospital, 1939-1941

	TOTAL CASES	CASES FOLLOWED	CURED CASES		FAILURES		DEFAULTERS	
			NO.	%	NO.	%	NO.	%
Sulfapyridine	87	62	57	92	5	8	25	28.8
Sulfathiazole	55	50	48	96	2	4	5	9
Sulfadiazine	57	55	51	93	4	7	2	3.5
Total cases	199	167	156	93.5	11	6.5	32	16.2

The results of treatment were uniformly good, no matter what the existing pathologic condition was at the time of beginning treatment. There were no complications in any cases during the period of treatment.

Analysis of the cured cases showed the average number of visits to the clinic before cure was complete to be eight for sulfapyridine, six for sulfathiazole, and eight for sulfadiazine.

In the entire series 11 cases were treatment failures. With sulfapyridine there were 5 failures. Failure in 2 cases was due to sensitivity to the drug that resulted in exfoliative dermatitis. The other 3 failures with sulfapyridine were patients who failed to cooperate during the period of treatment. They had coitus and alcoholic debauches during the early stage of treatment. With sulfathiazole there were 2 failures. These 2 patients failed to cooperate during the treatment period. With sulfadiazine 4 cases were treatment failures. It is interesting to note that 2 of these cases were syphilitic and were receiving mapharsen 0.06 Gm. per week at the same time that they were receiving sulfadiazine. The poor results in these two cases would bring up the question as to whether or not mapharsen has an inhibiting effect on the action of sulfadiazine. One of the failures had previously been a therapeutic failure with sulfanilamide and sulfathiazole. At the time that he began treatment with sulfadiazine he had a subacute anterior-posterior urethritis and prostatitis refractory to the two sulfonamides mentioned. The other failure did not cooperate.

The incidence of those who defaulted with sulfapyridine was 28.8 per cent, with sulfathiazole 9 per cent, and with sulfadiazine 3.5 per cent. The differences in default rate may be related to differences in the toxicity of the three drugs.

Toxic reactions were more commonly observed with sulfapyridine than with any of the other drugs. Seventy-five per cent of the patients treated with sulfapyridine complained of symptoms of toxicity, 11.5 per cent with sulfathiazole, and only 8.8 per cent with sulfadiazine. Three cases treated with sulfapyridine developed severe exfoliative dermatitis requiring hospitalization. These three cases were previously reported in detail.[8] Headache was the most common complaint with all three drugs. Complaints were severe among those receiving sulfapyridine, but minor among those taking sulfathiazole and sulfadiazine.

In the cured cases the time elapsing between the beginning of specific therapy and the cessation of discharge for sulfapyridine averaged three days, and ranged from two to eight days; for sulfathiazole, it averaged three days, and ranged from one to seven days; and for sulfadiazine it averaged four days and ranged from one to three days (see Table I).

The time that elapsed between the beginning of specific therapy and the first culture of the prostatic fluid which was negative for the gonococcus showed interesting differences among the three drugs. This period of time for sulfapyridine averaged fifty-one days, and ranged from fourteen to one hundred and sixty-two days; for sulfathiazole it averaged 28 days, and ranged from seven to eighty-eight days; and for sulfadiazine it averaged thirteen days, and ranged from three to forty-three days (see Table I).

The time elapsing from the disappearance of the symptoms of gonorrheal urethritis to the last culture of the prostatic fluid positive for the gonococcus is the period of time known as the so-called "carrier state." This period for sulfapyridine averaged seventeen days, and ranged from two to fifty days; for sulfathiazole it averaged seventeen days, and ranged from three to fifty-three days; and for sulfadiazine it averaged three days, and ranged from zero to eighteen days (see Table I).

Blood levels of the drugs, appearing with the dosages stated previously, were as follows: sulfapyridine, total, 1.6 to 9 mg. per 100 c.c.; sulfathiazole, free, 0.4 to 8.7 mg. per 100 c.c.; and total, 1 to 11 mg. per 100 c.c.; sulfadiazine, free, 1.5 to 10.4 mg. per 100 c.c.; and total, 2.1 to 11.5 mg. per 100 c.c. We did not find the blood levels of any prognostic value in regard to predicting toxic reactions or in determining the rapidity of cure.

The effect of the various sulfonamide derivatives studied on erythrocyte count, hemoglobin, leucocyte count, and differential count, was observed in 55 patients. In general, there were no significant changes.

Electrocardiographic studies were made on 51 patients before and after treatment with the drugs. There were no significant changes.

SUMMARY

A total of 199 patients were studied. Of this number 167 were followed to the completion of the study. Ninety-three and one-half per cent of these cases were cured. The rate of cure was 92 per cent for sulfapyridine, 96 per cent for sulfathiazole, and 93 per cent for sulfadiazine.* There is no significant statistical difference in these cure rates.

To effect a cure, the average dose required was for sulfapyridine 23.5 Gm. in eight days, for sulfathiazole 28 Gm. in eight days, and for sulfadiazine 17.5 Gm. in eight days.

*Five months' follow-up on the cases apparently cured by sulfadiazine revealed that of the 51 cases originally discharged as apparently cured, 8 had returned with reinfection. Each of these 8 cases had been observed for from twenty-eight to seventy-six days and had three to eight smears and cultures of the prostatic fluid negative for the gonococcus before being discharged as apparently cured. It is difficult to say definitely whether the reappearance of infection was due to recurrence of the old infection or to reinfection from a new contact of gonorrhea. Several cases gave a history of exposure. If we are to count the 8 cases of reinfection as treatment failures our percentage of apparent cures with sulfadiazine would be 78 per cent instead of 93 per cent originally reported.

Toxic reactions from the drugs occurred in 75 per cent of the cases treated with sulfapyridine, 11.5 per cent with sulfathiazole, and 8.8 per cent with sulfadiazine.

The average time for the discharge to cease in patients treated was three days with sulfapyridine, three days with sulfathiazole, and four days with sulfadiazine.

The average time for a bacteriologic cure, based on the appearance of the first negative culture of the prostatic fluid, was fifty-one days for sulfapyridine, twenty-eight days for sulfathiazole, and thirteen days for sulfadiazine.

The "carrier state" so-called (the period from the disappearance of symptoms to the last culture of the prostatic fluid known to contain living gonococci) averaged seventeen days for sulfapyridine, seventeen days for sulfathiazole, and three days for sulfadiazine.

The criteria of cure in this study were strict and included as the final provocative test two or more negative smears and cultures of the prostatic fluid.

CONCLUSIONS

From our studies it would seem that sulfadiazine is a valuable drug in the treatment of gonorrheal urethritis in the male because it has a high cure rate and a low incidence of toxicity; it causes the prostatic fluid to become free of living gonococci sooner than any of the other sulfonamides we used.

Sulfathiazole is very effective therapeutically and causes few toxic reactions.

Sulfapyridine would better not be used in the treatment of gonorrhea in the ambulatory patient. It is very effective therapeutically, but it gives rise to many and severe toxic reactions.

We wish to acknowledge the cooperation of Dr. Jefferson Clark, chief of the laboratories; Dr. Walter Crocker, chief of the division of clinical pathology; Dr. John Reinhold, chief of the division of biochemistry; and Dr. Thomas McMillan, chief of the division of cardiology of the Philadelphia General Hospital.

Sulfapyridine was furnished to us by Merck & Co., Rahway, N. J.

Sulfathiazole was furnished by the Maltbie Chemical Co., Newark, N. J.

Sulfadiazine was furnished through the courtesy of the Pneumonia Control Committee of the Philadelphia General Hospital, and the Nepera Chemical Co., Yonkers, N. Y.

REFERENCES

1. Pelouze, P. S.: Oral and Medical Treatment of Gonorrhea in the Male, J. A. M. A. **114**: 1878, 1940.
2. La Towsky, L. W., Knight, F., and Uhle, C. A. W.: The Treatment of Gonorrheal Urethritis in the Male With Sulfapyridine—A Study of 87 Cases, Am. J. Syph., Gonor. & Ven. Dis. **25**: 445, 1941.
3. Knight, F., Uhle, C. A. W., and La Towsky, L. W.: The Treatment of Gonorrheal Urethritis in the Male With Sulfathiazole—A Study of 55 Cases, J. Urol. **44**: 748, 1940.
4. La Towsky, L. W., Baker, Richard B., Knight, F., and Uhle, C. A. W.: The Treatment of Gonorrheal Urethritis in the Male With Sulfadiazine. An Analysis of 57 Cases, J. Urol. **46**: 89, 1941.
5. Uhle, C. A. W., La Towsky, L. W., and Knight, F.: A Comparison of the Results of Treatment of Gonorrheal Urethritis in the Male With Sulfapyridine and Sulfathiazole, J. A. M. A. **117**: 247, 1941.
6. Eisendrath, D. N., and Rolnick, H. C.: Urology, ed. 3, Philadelphia, 1934, J. B. Lippincott Co., pp. 177-180.
7. Bratton, H. C., and Marshall, E. K.: A New Coupling Component for Sulfanilamide Determination, J. Biol. Chem. **128**: 537, 1939.
8. La Towsky, L. W., Uhle, C. A. W., and Knight, F.: Severe Toxic Skin Reactions Following Sulfapyridine Therapy, M. Times **69**: 120, 1941.

PYELITIS WITH SEPTICEMIA CAUSED BY S. PARADYSENTERIAE*

EDITH HAYNES, PH.D., S. A. MANALAN, M.D., AND BEN B. HARVEY, M.D.
INDIANAPOLIS, IND.

COMPARATIVELY few cases of septicemia or of urinary tract infections due to paradysentery bacilli have been reported. Our case seems to be of special interest because both pyelitis and septicemia caused by *Shigella paradysenteriae* complicated the post-partum course of the patient. The same organism was isolated from two stool specimens, although no diarrheal symptoms were observed.

Approximately 30 cases have been recorded in which a septicemia has been attributed to a dysentery bacillus. In 1926 Ashworth and others reviewed the literature on the occurrence of septicemia in cases of bacillary dysentery. He listed 24 cases, including the one which he reported, but among these were several which were not well proved. Rothman[2] in 1936 added one case to this list, and in 1937 one of us[3] reported two fatal cases of paradysentery in infants with positive blood cultures. Dodd and Swanson[4] in 1938 reported three cases of paradysentery from one of which a positive blood culture was obtained before death, and from the other two cases post mortem.

Fourteen cases of infection of the urinary tract by paradysentery bacilli had been recorded when the literature was reviewed by Neter[5] in 1937. In two of three cases which he added to this group, a paradysentery bacillus was isolated from the stool as well as the urine; in the other case apparently only from the urine. No blood cultures were made. In August, 1940, we had in our hospitals two cases, and in December, 1940, there were also two cases of urinary tract infection due to paradysentery bacilli. The blood cultures of the first two, and one of the second pair, all of which were adults, were negative, while none was taken from the fourth patient, who was a child.

Two cases reviewed by Neter,[5] that reported by Calalb and Ionesca and that reported by Cheatham, were cases of pyelonephritis complicating pregnancy.

CASE REPORT

D. R., aged 41 years, para 0, gravida iii, was admitted to the William H. Coleman Hospital on July 5, 1940, when her pregnancy was approximately at term. She complained of epistaxis of three days' duration. Her physician had detected hypertension at the time of her first visit at the sixth month. Five and again two years previously, she had had spontaneous abortions at six months. Her last pregnancy was known to be complicated by a systolic blood pressure of 220. She gave a history of rheumatic fever as a child. At the age of 3 she had had a severe diarrhea, but no definite diagnosis was made. She had been employed as a nurses' aide in a mental institution from the age of 30 to 38. During this time she had suffered from a diarrhea of two days' duration. Many patients she had cared for had had diarrhea of an undetermined origin.

Physical examination upon admission revealed a markedly obese white female. The abdomen was enlarged to the size of a full-term pregnancy. Fetal heart sounds were heard.

*From the Departments of Clinical Laboratories and of Obstetrics, Indiana University Medical Center.
Received for publication, May 31, 1941.

The retinae exhibited tortuosity of the retinal vessels, several small exudates, crossing phenomenon, and a vein-artery ratio of 2 to 1. A rough, low-pitched systolic murmur was heard loudest at the upper end of the sternum and the aortic region. The blood pressure was 220/110. There was slight edema of the lower extremities.

On admission the urine contained 0.521 per cent albumen, one granular cast per high-power field, and ten to twelve pus cells; blood counts were essentially normal. Eight hours following admission, the membranes ruptured spontaneously, and uterine contractions promptly ensued. Approximately ten minutes later no fetal heart sounds were heard. No abnormalities were found on vaginal examination. Labor was terminated at the end of twelve hours by the delivery of a full-term stillborn male infant. There was evidence of compression of the umbilical cord due to the peculiar manner in which the cord was wrapped about the infant's abdomen and both thighs. Ten hours' post partum the patient was seized by a severe pain in the right chest which markedly impaired respiratory movements. Examination revealed a friction rub in the area of pain, and a blood pressure reading of 165/100. Symptomatic treatment was instituted consisting of morphine and nasal oxygen. Four hours later a similar episode occurred, involving the lower portion of the left chest. Her pulse was of poor quality and her temperature was 100° F. rectally. A bedside flat plate of the chest was obtained, which showed only a haziness in the lower lung fields. The following day her condition appeared to be slightly improved; orally, her temperature was 102° F. On the third post-partum day her temperature rose to 104° F., and she had a chill. Her complaints then were referable to the pain in the chest, and no new physical findings were obtained. Another flat plate of the chest showed a clearing of the previously reported congested areas. From the fourth to the ninth days post partum, inclusive, she maintained a low-grade temperature elevation. On the tenth post-partum day the temperature which rose to 104.8° F. was accompanied by a chill and increased pain along the right costal border. On the eleventh post-partum day the temperature rose to 103.4° F. There were many clumps of pus cells and 1+ albumen in catheterized specimens of urine obtained these two days. A tentative diagnosis of pyelitis was made. A blood culture was obtained which yielded a gram-negative bacillus, which had typical cultural characteristics of the paradysentery bacillus and was agglutinated by Hiss-Strong antiserum to a dilution of 1:160.

The patient was given sulfanilamide therapy by mouth, following which there was general improvement in her condition. Examination of the fundi on the twelfth post-partum day revealed the presence of a recent flame-shaped hemorrhage in the right fundus. From the fourteenth to the twenty-eighth post-partum days the low-grade temperature elevation persisted, following which her temperature returned to normal and remained so thereafter for a period of twenty-two days. Her blood pressure stabilized at 140 systolic and 100 diastolic. Urea clearance was 51 per cent. X-ray studies made of the chest, urinary tract, gastrointestinal tract, and biliary tract failed to add any information to this case. A diagnosis of toxemia of pregnancy upon a vasculorenal basis, pulmonary embolism, septicemia due to a paradysentery bacillus, and post-partum pyelitis was made.

A gram-negative bacillus identical with that obtained from the blood was obtained in pure culture from the urine on July 19, 1940, and was isolated from the stool on July 23, 1940. A second stool specimen on July 26, 1940, yielded the same organism. Blood cultures made on July 19, August 3, August 4, and August 6, 1940, were all negative.

Blood drawn from the patient on July 23, 1940, agglutinated *S. paradysenteriae* Strong at 1:160 dilution and *S. paradysenteriae* Hiss at 1:40 dilution. It agglutinated the organisms obtained from the blood, the urine, and the stool cultures at a dilution of 1:5,120. On August 20, 1940, the patient's blood serum agglutinated both Hiss and Strong dysentery bacilli at 1:160, and the organism isolated from the patient at 1:2,560 dilution.

Gram-negative bacilli, which seemed to be rough variants of the paradysentery bacillus, were isolated from the urine and stool on August 20, 1940. These bacilli gave typical fermentation reactions in the test substances used, but in spite of repeated efforts, smooth strains, which would be suitable for agglutination tests, were not obtained from the cultures.

It seems possible that this patient was a carrier of the paradysentery bacillus and, since her resistance was reduced, an acute infection with the organism developed. The probable persistence of the organisms in the rough form after the acute symptoms had disappeared is of interest in this connection. Neter[5] concluded from his study of urinary tract infections caused by paradysentery bacilli, that these infections may occur during or following an attack of intestinal dysentery or in patients without clinical evidence or history of dysentery. It seems possible that the organism may persist in the genitourinary or intestinal tract after mild attacks of dysentery that the patient does not remember.

CONCLUSION

A case of septicemia and urinary tract infection due to *Shigella paradysenteriae* complicating the post-partum course of a patient has been reported. So far as we have been able to determine this is the first case to be reported of a paradysentery bacillus infection of the urinary tract with a positive blood culture.

REFERENCES

1. Ashworth, O. O., and Upchurch, R. W.: Bacillary Dysentery—Report of a Case, With Laboratory Discussion by Straus, A. H., Virginia M. Monthly **53**: 359, 1926.
2. Rothman, P. E.: Dysentery Bacillemia, J. Pediat. **9**: 166, 1936.
3. Haynes, E.: A Bacteriological Study of Forty Cases of Dysentery in Infants and Children, Including Two Cases of Flexner Bacillus Septicemia, J. Infect. Dis. **60**: 251, 1937.
4. Dodd, K., and Swanson, H.: Dysenteric Bacteremia, Am. J. Dis. Child. **56**: 1082, 1938.
5. Neter, E.: Infections of the Urinary Tract Due to Bacterium Dysenteriae, J. Infect. Dis. **61**: 338, 1937.

SYNDROME SIMULATING DIABETES INSIPIDUS IN DOGS INDUCED BY DESOXYCORTICOSTERONE ACETATE*

Clinical Observation of Syndrome With Addition of Tetany

Robert C. Moehlig, M.D., and Louis Jaffe, M.D., Detroit, Mich.

RECENTLY Ragan and associates[1] reported that with daily 25 mg. doses of desoxycorticosterone acetate injected into dogs for a period of six weeks, they were able to induce a syndrome of polydipsia and polyuria. Pitressin did not influence the syndrome, thus differing from true diabetes insipidus. We obtained similar results in dogs, using smaller doses in varying concentrations (5 and 10 mg.) over a period of four months. Some of the material used was dissolved in propylene glycol.†

Two of 5 dogs used developed the syndrome of polydipsia and polyuria, and one of these died.

Muscular weakness and lack of coordination were prominent features in all 5 dogs. Ragan and associates observed this in their dogs, and stated that it had previously been observed by Kuhlmann and others.

*From the Department of Internal Medicine, Harper Hospital, Detroit.
†The material was kindly furnished by Dr. Max Gilbert, of the Schering Corporation.
Received for publication, May 31, 1941.

The specific gravity of the urine in our polyuric dogs showed a gradual decrease toward 1.000. The intake of fluids was not accurately measured but all the experimental animals showed a marked increase in fluid intake.

Two dogs had outstanding symptoms of polydipsia and polyuria, being constantly thirsty and drinking large quantities of water. These two dogs died, while the others showed disappearance of the syndrome when the dosage was reduced to 5 mg. per day.

Soon after the syndrome was noted in the dogs, a male patient suffering from myasthenia gravis and in a relapse had a total pellet implantation of 1,300 mg. of desoxycorticosterone acetate. One pellet of 150 mg. was purposely crushed. Within two weeks he developed a very marked polydipsia and polyuria. There was an increase of blood pressure from systolic 110 and diastolic 68 to systolic 138 and diastolic 92.

Coincident with the polydipsia and polyuria, there developed symptoms of tetany—a positive Chvostek sign and Trousseau's phenomenon was easily brought out during the reading of the blood pressure. He had spontaneous tetany of his hands several times during the day. Paresthesia of the extremities was also present. The serum calcium was 8 mg., and phosphorus was 3.93 mg. per 100 c.c. of blood. The electrocardiogram showed a prolonged RT-interval of 0.32 like that seen in hypoparathyroidism.

It is of interest that soon after the implantation the patient had a return of muscular strength, but when the polydipsia, polyuria, and tetany set in, symptoms of muscular weakness became marked. This was in distinct contrast to the striking improvement following the first implantation of the pellets. This muscular weakness differed from the original myasthenia gravis symptoms in that there was no recovery with rest; nor was there any speech difficulty. Further details of this will be given elsewhere. We know from our experimental data, as well as that of Ragan and associates, that this muscular weakness was in all likelihood due to overdosage of the drug.

The symptoms mentioned continued for two months, and for the last three months the patient states that he has never felt as well since the onset of his illness in October, 1939. A mild degree of polydipsia and polyuria are still present, but the tetany and paresthesia have disappeared. To our knowledge, this is the first instance in which the syndrome of polydipsia, polyuria, and tetany with paresthesia has been produced in a human being by overdosage with desoxycorticosterone acetate.

The patient has repeatedly emphasized that the injections of the drug, while seemingly holding the disease stationary, do not compare with the relief obtained by implantation of the pellets. This is also the observation of Thorn.[2]

Our experiments confirm the findings of Ragan and his associates. They made careful electrolytic studies of their dogs in which they induced polydipsia and polyuria by giving daily injections of 25 mg. of desoxycorticosterone acetate. While the symptoms were similar to diabetes insipidus, they noted that pituitrin was relatively ineffective, and fluid restriction did not cause dehydration in the animals. The converse is true in diabetes insipidus. They are of the opinion, therefore, that the condition which they described is primarily a thirst, and only secondarily a polyuria. Why this should be is not known, although speculative possibilities naturally present themselves.

Sections of various organs, such as the thyroid, adrenal, testes, ovaries, kidney, spleen, and muscle were all normal, except in one dog which showed deglycogenation of the liver. Separate sections of the hypothalamic area were made of the two dogs showing the polydipsic and polyuric syndrome. Unfortunately, the pituitary was lost during preparation for sectioning. The remainder of the pathologic report follows:

"In sections of one of the blocks there is a region in which the leptomeninges are infiltrated with plasma cells and in this region an adventitial infiltration of the intracerebral vessels is also seen. The infiltrating cells consist almost exclusively of plasma cells forming large coats around the lumen of the vessels. There seems to be an increase of blood vessels. However, this picture could be due to a more pronounced marking of pre-existing vessels because of adventitial infiltration. There is no visible adventitial endothelial proliferation. The region in which this definite pathologic finding was seen belongs to some part of the hypothalamus. It could not be determined which hypothalamic nucleus particularly is involved. The pituitary was not included in the blocks."

Summary: "The finding represents a localized encephalitic reaction showing a plasmacellular infiltration of the leptomeninges and the adjacent intracerebral blood vessels. Whether or not this encephalitic reaction is a part of a more generalized process cannot be determined because of lack of material."*

These findings suggest certain correlations with the clinical picture of hypertension, polydipsia, and polyuria. This is based on the known physiology of the hypothalamus in its influence on blood pressure and water balance. Further studies along these lines are suggested by these data.

One of the dogs was pregnant and received the desoxycorticosterone acetate injections daily for six weeks and bore a litter of seven, only two of which were viable. One of the latter died within a week. One may conclude from this that the drug was probably responsible for the fetal deaths. We are not prepared to say as to the mechanism by which this was produced.

Selye's[3] work is of interest in this connection. He concluded that his experimental evidence indicated that both in the mouse and in the rat desoxycorticosterone acetate causes marked involution of the adrenal cortex. He stated that the atrophy of an endocrine gland caused by the administration of an excess of the hormone or hormones which it produces is regarded as the exact antithesis of the compensatory hypertrophy elicited by the hormone deficiency occasioned by partial extirpation of such a gland. For this mechanism of readjustment, Selye suggests the term "compensatory atrophy."

He is of the opinion that this is of clinical importance and says that, for example, the use of desoxycorticosterone acetate (a substance which is practically devoid of androgenic activity) in cases of adrenogenital syndrome in which there is an excess production of androgen in the adrenal may prove useful in inhibiting the faulty endocrine secretion of the cortex.

This would not be without its dangers, however, since in this syndrome hypertension is already present, and the known hypertensive effects of the desoxycorticosterone acetate would add fuel to the fire. Certainly the danger of

*We wish to thank Dr. Gabriel Steiner from the Department of Pathology, Wayne University, for the pathologic report.

vascular cerebral, and cardiac accidents, is to be considered, and this might occur before "compensatory atrophy" had set in.

The drug, no doubt, is a very useful one but must be used with caution, since it may give rise to dangerous, toxic symptoms.

SUMMARY

A syndrome of polydipsia and polyuria was induced in two dogs by comparatively large doses of desoxycorticosterone acetate. Profound muscular weakness was noted in the dogs so that they were unable to co-ordinate their muscles.

A pregnant dog which had received the drug for a period of six weeks gave birth to a litter of 7, 5 of which died, one lived for a week, and the remaining one survived.

Sections of the hypothalamus showed a localized encephalitic reaction with a plasmacellular infiltration of the leptomeninges and adjacent intracerebral blood vessels. Whether or not this encephalitic reaction is a part of a more generalized process cannot be determined because of lack of material. The suggestion was made that the clinical picture of polydipsia, polyuria, and hypertension might be understood by changes occurring in the hypothalamus.

A patient with myasthenia gravis, who had an overdosage of the drug by implantation of the pellets of this material and the crushing of one pellet, showed the syndrome of polydipsia and polyuria. Muscular weakness, such as was noted in the experimental animals, was also present. In addition, he had signs and symptoms of tetany, which we believe is a new symptom of overdosage.

REFERENCES

1. Ragan, C., Ferrebee, J. W., Phyfe, P.: Atchley, D. W., and Loeb, R. F.: Syndrome of Polydipsia and Polyuria Induced in Normal Animals by Desoxycorticosterone Acetate, Am. J. Physiol. **131**: 73, 1940.
2. Thorn, Geo. W.: Treatment of Addison's Disease, J. Clin. Endocrinol. **1**: 76, 1941.
3. Selye, Hans: Compensatory Atrophy of the Adrenals, J. A. M. A. **115**: 2246, 1940.

964 Fisher Building
1114 Eaton Tower

THE EFFECT OF PITRESSIN ON RENAL CIRCULATION AND URINE SECRETION*

KHALIL G. WAKIM, M.D., J. F. HERRICK, PH.D., EDWARD J. BALDES, PH.D., AND FRANK C. MANN, M.D., ROCHESTER, MINN.

CONSIDERABLE work on human subjects and on various species of animals has been done in an effort to determine the action of pitressin on the circulation of blood in the kidney as well as on urine formation, and to explain the mechanism of antidiuresis. Diverse methods have been employed, and pituitary extracts containing various amounts of impurities have been administered. Consequently, considerable data have accumulated which manifest equivocal interpretations and contradictory conclusions. This paper is a presentation of an attempt to demonstrate the alterations in the glomerular tuft of capillaries and other vessels in the frog kidney, as seen under the microscope, and the changes in renal blood flow in the dog kidney under the influence of pitressin when administered through the various possible routes under similar conditions. The only preparation used for the work reported in this paper was pitressin in ampoules (1 c.c. = 20 units).

A. EFFECT OF PITRESSIN ON THE GLOMERULAR CIRCULATION IN THE FROG KIDNEY

Adolph[1, 2] employed pituitrin (solution of posterior pituitary) as one of the methods of decreasing urine formation in the frog kidney. He observed that all the methods that diminished the production of urine decreased the circulation of the blood either locally or generally. He, therefore, suggested that these effects on the circulation account for the decrease in urine formation. By subcutaneous injection of pituitary extract into the frog *(Leptodactylus ocellatus)*, Biasotti[3] found that in twenty-four hours the normal frogs had an average increase of 15.6 per cent in weight, and those with ligated ureters had an average increase of 26 per cent. He stated that Brunn[4] did not consider the effect to be produced by the kidney, because even in nephrectomized frogs the administration of pituitary extract causes a greater increase in weight than in the controls. Rey[5] injected posterior pituitary extract (Pituilobine Byla A or B) into the abdominal lymph sac of frogs *(Rana temporaria)*, and observed a great diminution of urine formation for two or three hours after injection. He suggested that the posterior lobe extract modifies the three factors that take part in the regulation of water exchange in the body: namely, (1) the excretion by the kidney, (2) the retention by the tissues, and (3) the absorption by the skin.

On the other hand, the work of Collin and Drouet[6] casts doubt on the water retention action of the posterior pituitary extract. They injected the extract

*From the Mayo Foundation, the Mayo Clinic, Rochester.
Received for publication June 9, 1941.

(Choay No. 2 and No. 4) into frogs and compared their weight changes, for forty-eight hours after injection, with those of controls under the same environmental conditions. Their findings indicated weight changes in those injected with extracts of the posterior pituitary lobe similar to the changes in the controls. Burgess, Harvey, and Marshall[7] accumulated evidence which enabled them to conclude that pitressin and pituitrin exert no antidiuretic action in the frog, but that in mammals the antidiuretic action of these substances is "due to a stimulation of water reabsorption by the thin segment of the loop of Henle." From his work on water interchange in frogs and their weight increases under the influence of pitressin, Steggerda[8] suggested the probability that pitressin causes an increase in skin permeability, possibly by acting directly on the circulatory system or by some change in the melanophores. He expressed the opinion that the antidiuretic effects of pitressin do not play a major role in the weight increases of the frogs. Richards and Schmidt[9] observed that small amounts of pituitrin administered intravenously increased the number of active glomeruli, and also the number of active capillaries in the tuft of a single glomerulus in the frog kidney; but both were decreased under the influence of large doses.

Method.—For our observations on the circulation in the frog kidney we modified the method of illumination described by Knisely.[10, 11] The experiments were made on anesthetized male frogs *(Rana pipiens)* whose weights ranged from 25 to 45 Gm. Anesthesia was produced by injecting into the dorsal lymph sac 0.1 c.c. of a 25 per cent solution of urethane per 10 Gm. of body weight. The abdomen of the frog was opened by a paramedian incision, carefully avoiding the big vessels, and the edges of the incision were gently cauterized to prevent loss of blood. The animal was laid on a block of cork with frog-Ringer's solution gently flowing over the exposed viscera to prevent drying. Water was kept trickling around the intact body of the frog to dilute the Ringer's solution in contact with the skin and to prevent dehydration. The kidney was protected from the heating effect of the light, transmitted through the quartz tubing, by a constant stream of Ringer's solution flowing through the bore in the delivery tip introduced retroperitoneally, dorsad to the kidney. Such a preparation could be kept functioning normally, without any signs of hemorrhage or other disturbances, for at least nine hours. Healthy frogs were used, and the normal kidney circulation in several microscope fields was carefully studied before any observations on the effects of pitressin were made. Amounts of pitressin ranging from 0.01 to 0.1 c.c. were injected either into the lymph sacs or intravenously into the anterior abdominal vein, or were applied directly through a very fine needle to the microscope field under observation. A stop watch was used to record the time when the drug was administered, when any effect was observed, and when the affected parts returned to their original condition previous to the administration of the drug.

Results.—When pitressin was administered by direct application of 1 or 2 drops over the microscope field under observation, it was found that in three to ten seconds undiluted pitressin produced cessation of activity in all glomeruli in the field. In some glomeruli the afferent arterioles contracted to disappearance during the maximal effect, and whatever blood cells were in the glomerular tuft of capillaries remained motionless until renewed activity gradually took

place. Such renewed activity would occur in a period ranging from two to ten minutes. However, not all the glomeruli ceased functioning at once, nor did they become active again at the same time. The afferent arterioles of some glomeruli contracted enough to prevent passage of blood cells while plasma still passed and gradually forced the corpuscles out of the glomeruli, thus leaving the glomerular tuft of capillaries free of blood cells until renewed activity took place. The blood flow in the other vessels of the kidney area under observation became so slow after application of pitressin that for a period of several minutes no axial or peripheral streams could be demonstrated, and the cells, white and red, could be easily identified in the slow stream throughout the lumina of the vessels. Dilutions of pitressin up to 1:100, when directly applied in 2 drops over the area under observation, gave the same changes but for much shorter duration. Higher dilutions gave no detectable change.

Injection of undiluted pitressin in amounts ranging from 0.05 to 0.1 c.c. into the lymph sac produced within two minutes a noticeable slowing of blood flow throughout the kidney areas under observation. Changes similar to those described under direct application of the drug were noticed, except that the glomeruli, instead of showing complete cessation of blood flow, showed very sluggish flow through their capillary tufts. This lasted for at least twenty minutes, and often for more than half an hour. Dilutions up to 1:200, when given in amounts of 0.1 c.c. into the lymph sac, produced a slowing of the blood stream in the various vessels of the kidney area, which was much less marked and shorter in duration than that produced by the undiluted dose.

The injection of 0.05 c.c. to 0.1 c.c. of undiluted pitressin into the abdominal vein of the frog produced, within twenty seconds, complete cessation of activity of all the glomeruli under observation. Some glomeruli became completely blanched and had no blood cells in their tuft of capillaries whatsoever, while other glomeruli had their capillaries packed with motionless red and white blood cells. This cessation of activity lasted for periods varying from two to eight minutes, after which recovery began to take place in the glomeruli at different times. The other vessels in the area showed marked slowing of the blood stream to such an extent that the flow became jerky, and in some vessels it wavered back and forth without much forward progress. The slowing of the blood stream in the kidney tissue under observation lasted from twenty to forty-five minutes. Dilutions up to 1:500 gave such effects as already described, but the duration generally varied inversely with the dilution. Dilutions higher than 1:500 produced no detectable changes. No matter what route was used for administration, undiluted pitressin produced various degrees of anemia of the area under observation as a result of vascular constriction.

B. EFFECT OF PITRESSIN ON RENAL BLOOD FLOW AND URINE SECRETION IN THE DOG

In a clinical investigation on human subjects, Stead, Kunkel, and Weiss[12] found that the intramuscular administration of pitressin produced a greatly reduced blood flow in the hands as well as abdominal cramps and ashen pallor. In their study on the trained dog, Geiling and co-workers[13] reported a marked and prolonged decrease of blood flow in the femoral and carotid vessels (arteries

and veins) following the intravenous administration of pitressin. However, in a few observations on the renal artery they observed a transient decrease followed by a prolonged and marked increase. Grindlay and two of us (Herrick and Mann[14]) reported a marked decrease in the blood flow of the spleen following the intravenous injection of 0.05 c.c. of pitressin into dogs. Essex and his co-workers[15] reported that the intravenous administration of pitressin into trained dogs produced a decrease from 64 to 84 per cent in the coronary blood flow and reduced the heart rate.

Handovsky and Samaan,[16] using the Rein thermostromuhr on both anesthetized and unanesthetized dogs, observed an initial reduction followed by a prolonged increase in renal blood flow under the influence of large doses of posterior pituitary extract administered intravenously. They used various commercial preparations of posterior pituitary extracts. Janssen and Rein[17] determined the renal blood flow of decerebrate dogs under the influence of fresh posterior pituitary extract. They concluded that the process of antidiuresis was not accompanied by any alterations in the renal blood flow. Walker and co-workers[18] injected pituitrin subcutaneously into unanesthetized rabbits and dogs, and determined the renal blood flow during the development of the antidiuresis. They observed very slight decrease in renal blood flow in some experiments, and an apparent marked increase in three of ten experiments. They stated that decrease in renal blood flow does not play any part in the antidiuresis in either dog or rabbit. Unpublished observations by A. E. Livingston and one of them, reported in a footnote, indicate a decrease in the size of the dog's kidney observed roentgenographically during pituitrin antidiuresis. They attributed this decrease to changes in tubule volume rather than to a decrease in the size of the vascular bed. Their observations on creatinine clearances indicated no consistent reduction under the effect of pituitrin, and they concluded that changes in the glomerular filtrate are not the mechanism of the antidiuresis.

In their work on dogs, Underhill and Pack[19] found that intravenous administration of pituitrin caused marked dilution of the blood, and that exclusion of the kidneys by mass ligature at the hilus previous to the injection of pituitrin did not modify the hydremia produced by the pituitrin. They considered the hydremia and the antidiuretic action to be due to some unknown action on capillary permeability. From his work on the isolated, perfused kidney of the dog, Verney[20] concluded that the pituitrin-like secretion picked up by the blood passing through the head inhibited the polyuria of the isolated kidney and decreased the renal blood flow. Richards and Plant[21] determined renal blood flow by the method of Barcroft and Brodie[22] in dogs, rabbits, and cats under urethane anesthesia after intravenous administration of small doses of pituitrin. They obtained a decrease in renal blood flow in dogs and rabbits but not in cats.

Method.—The observations on the renal blood flow were made by the thermostromuhr method of Rein, which was modified by Essex and two of us (Baldes and Herrick[23, 24]). Direct current thermostromuhr units were carefully calibrated before and after application. The calibrations were made and checked in such a way that the deflections of a sensitive moving coil galvanometer could be translated into cubic centimeters of blood flow per minute.

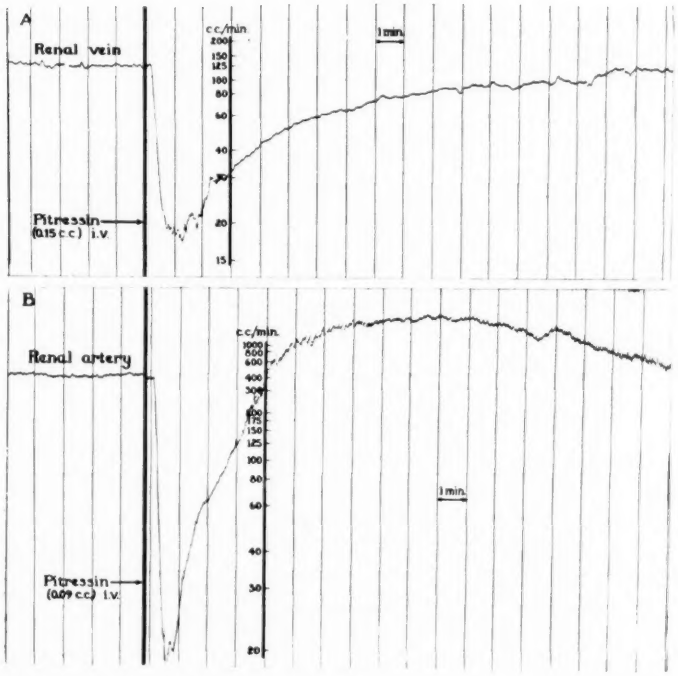

Fig. 1.—Effect of pitressin on the renal blood flow in the trained dog. *A* demonstrates the sudden and marked decrease of blood flow in the renal vein under the influence of intravenously administered pitressin. More than eighteen minutes elapsed before the blood flow returned to the preinjection level. *B* demonstrates the effect of a small dose of pitressin given intravenously on the blood flow in the renal artery. This was markedly decreased, but within five minutes the flow increased beyond the preinjection level.

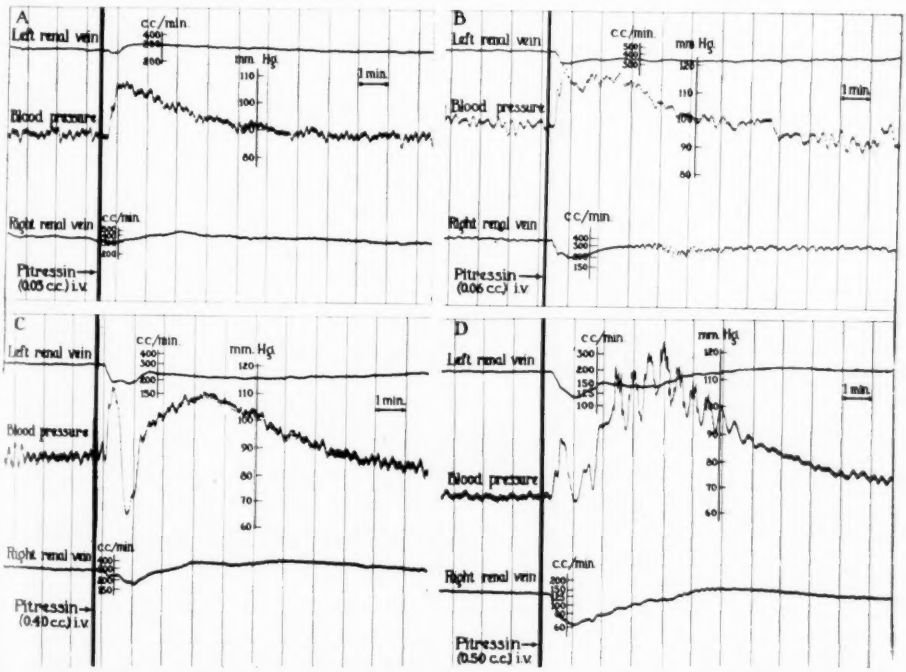

Fig. 2.—Effect of various doses of pitressin on the carotid blood pressure and on the blood flow in the right and left renal veins of the anesthetized animal (pentobarbital sodium).

Three groups of healthy dogs were used for investigating this phase of the problem. The anesthetic used on the first group was pentobarbital sodium, which was slowly administered intravenously in amounts of 25 mg. per kilogram of body weight. The kidney was well exposed through a midline incision of the abdomen, and the renal artery or vein was gently dissected and carefully cleaned, avoiding injury to any of the nerves in the hilus. A calibrated unit was snugly fitted around the renal vessel and conveniently anchored to avoid any interference or turbulence in the blood flow of the vessel used. The same technique was used in applying another unit on the vessel of the second kidney. In some experiments a unit was placed on the common iliac artery or vein instead of one of the kidney vessels in order to permit simultaneous recording of the effect of pitressin on the blood flow of the kidney as well as of the lower extremity.

TABLE I

PITRESSIN: A COMPARISON OF SUBCUTANEOUS, INTRAMUSCULAR, AND INTRAVENOUS INJECTIONS

DOG NO.	WEIGHT (KG.)	OBSERVATIONS MADE	DOSE (UNITS PER KG.)	MODE OF INJECTION	PERCENTAGE DECREASE IN RENAL BLOOD FLOW FOLLOWING INJECTION
1	13.3	During pentobarbital sodium anesthesia	0.6	Intravenous	66
			0.6	Intramuscular	6
			0.6	Intramuscular	8
			0.6	Subcutaneous	8
			0.6	Subcutaneous	8
			0.6	Intravenous	75
2	19.3	During chloralosan anesthesia	0.6	Intravenous	48
			0.6	Intramuscular	3
			0.6	Subcutaneous	1
			0.6	Subcutaneous	9
			0.6	Intramuscular	7
3 Trained dog	17	Second day after operation	0.3	Subcutaneous	7
			0.075	Intravenous	38
			0.3	Intramuscular	10 (increase)*
			0.15	Intravenous	47
		Third day after operation	0.15	Intravenous	55
			0.45	Intramuscular	11 (increase)*
			0.30	Intravenous	51
4 Trained dog	21.7	Second day after operation	0.15	Intravenous	96
		Third day after operation	0.075	Intravenous	96
			0.3	Intramuscular	30
			0.3	Subcutaneous	21

*In two instances the flow was increased slightly by intramuscular injections in Dog 3.

NOTE: Intravenously administered, pitressin produces consistently a decrease in renal blood flow; intramuscularly, it gives variable results; subcutaneously, it usually has no effect on the renal blood flow.

Both ureters were isolated and cannulated at their entrance into the bladder. Y-shaped glass tubing was connected to the ureteral cannulas to lead the urine into a common exit for recording the rate of urine flow. The femoral vein was exposed for intravenous administration, and the carotid artery was cannulated for recording blood pressure. Heparin was used as anticoagulant in the system for optical recording of the carotid blood pressure. The abdomen was closed, leaving between the sutures the tubing of the ureteral cannulas and the flexible, rubber-insulated lead wires of the units. The two galvanometer deflections translating the blood flows in the two kidneys and the optical record of

the blood pressure were all photographed on slowly moving photosensitive paper, thus furnishing permanent records of control values, and of any changes during and after the administration of pitressin for any length of time desired.

The same technical procedures and observations mentioned in the preceding paragraph were made on another group of dogs which were anesthetized by slow intravenous administration of chloralosan—100 mg. per kilogram of body weight, dissolved in warm Ringer's solution.

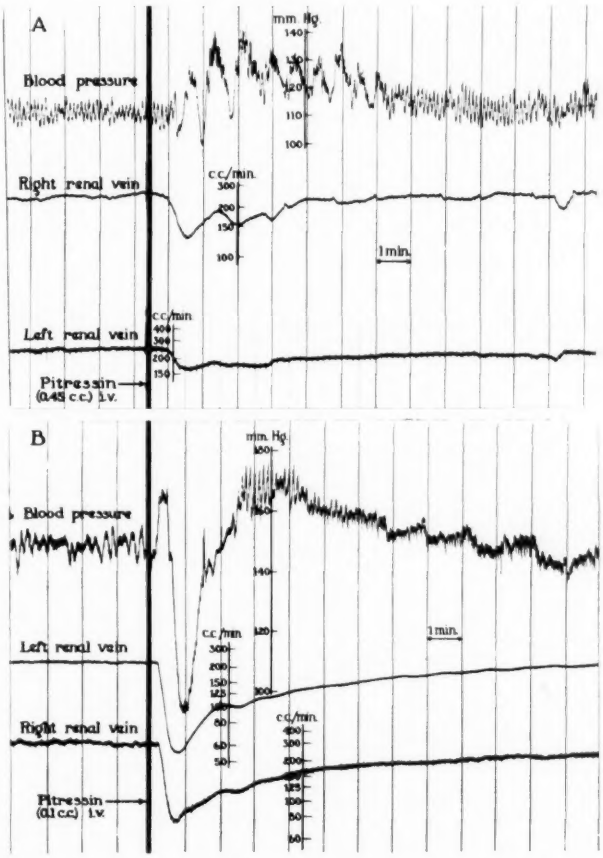

Fig. 3.—Effect of pitressin under different anesthetics: *A*, chloralosan; *B*, pentobarbital sodium. Under chloralosan, the injection of pitressin, in spite of the much larger dose, gave a much smaller decrease in renal blood flow than did pentobarbital sodium.

For the purpose of avoiding the influence of anesthesia, another series of experiments were performed on dogs that had been trained to lie quietly on the table. On a day prior to the experiment, the dogs were etherized and, with the usual aseptic technique, the renal vessel was exposed and a calibrated unit was applied as previously described. The lead wires of the unit were exteriorized, and the abdominal incision was carefully sutured according to surgical principles. Observations on renal blood flow were made after the dog had recovered from the operation. After a few days of blood flow studies, the femoral artery was exposed under local anesthesia, and the blood pressure was optically recorded simultaneously with the renal blood flow on the same chart.

TABLE II

EFFECT OF SUCCESSIVE DOSES OF PITRESSIN (GIVEN INTRAVENOUSLY) ON BLOOD FLOW IN RENAL VEINS (WEIGHT OF DOG, 13.2 KG.)

DOSE (UNITS PER KG.)	PERCENTAGE DECREASE IN BLOOD FLOW	
	LEFT RENAL VEIN	RIGHT RENAL VEIN
0.15	67	70
0.15	66	58
0.15	75	72
0.15	63	65
0.075	50	49
0.075	54	57

TABLE III

EFFECT OF PITRESSIN ON URINE FORMATION IN ANESTHETIZED DOGS

DOG NO.	WEIGHT (KG.)	DOSE (UNITS PER KG.)	MODE OF INJECTION	URINE, DROPS PER MINUTE		DURATION OF EFFECT (MINUTES)
				BEFORE INJECTION	AFTER INJECTION	
1	13.3	0.6	Intravenous	6	3	6
		0.6	Intramuscular	4	4	No effect
		0.6	Subcutaneous	6	6	No effect
2	19.3	0.6	Intravenous	6	1	9
		0.6	Intramuscular	13	9	30
		0.6	Subcutaneous	12	12	No effect
5	13.8	0.4	Intravenous	3	1	8
		0.4	Intramuscular	2	2	No effect
		0.4	Subcutaneous	1	1	No effect
		0.4	Subcutaneous	1	1	No effect
6	12.0	0.5	Intravenous	5	1	8
		0.5	Intramuscular	3	2	60
7	18.8	0.3	Intravenous	4	1	7
		0.5	Intramuscular	11	10	60
8	22.0	0.65	Intravenous	4	1	8
		0.65	Intramuscular	3	3	No effect
9	15.4	0.5	Intravenous	5	1	10
		0.5	Intramuscular	3	3	No effect

NOTE: Intravenous administration of pitressin immediately causes anuria followed by oliguria for about six to ten minutes. Intramuscularly it produces slight oliguria, but subcutaneously it does not have any effect on the urine formation in the anesthetized animal.

Results.—When given intravenously, pitressin has a pronounced effect on the renal blood flow in the trained dog, that is, a marked decrease (Fig. 1). This effect depends on the amount of the drug injected (Fig. 2). The decrease in flow is less pronounced when the dog is anesthetized and seems to vary with the kind of anesthetic used (Fig. 3).

When pitressin is given either intramuscularly or subcutaneously, its effect is less marked. In contrast to the intravenous injection, the subcutaneous injection produces no significant change in either the renal blood flow or in the systemic blood pressure. Intramuscular injections produce slight but variable changes in renal flow (Table I).

Some investigators have expressed the opinion that subsequent injections of pitressin do not have as marked an effect as the first injection. We have not been able to confirm this. Successive injections were never made, however, until the renal blood flow had returned to the preinjection value. Under these circumstances successive injections caused a decrease similar to that of the first (Table II).

Concerning the effect of pitressin on blood pressure, we wish to call attention to the fall in blood pressure following the immediate transient rise (Figs. 2*C* and 3*B*) which sometimes takes place previous to the prolonged moderate increase.

The decrease in renal blood flow is not maintained over as long a period as that in the femoral or carotid arteries. Another distinguishing characteristic is that the initial decrease in renal flow is followed sometimes by a prolonged and marked increase in the trained dog (Fig. 1*B*).

The well-known decrease in urine flow occurred following intravenous injections of pitressin (Table III).

SUMMARY

By means of transillumination, direct observations were made on the effect of pitressin on the glomerular tuft of capillaries and other vessels in the frog kidney. Various dilutions of pitressin were applied directly to the illuminated area of the kidney, or were injected into the lymph sac or into the abdominal vein of the frog under urethane anesthesia. Depending on the amount and dilution used, pitressin produced cessation of the circulation in the glomerular tuft of capillaries for a fraction of a minute to several minutes, and slowed the circulation in the other vessels to such an extent that no axial stream was observed, and the white and red corpuscles could be easily distinguished in the slowly moving stream within the lumen of the vessel.

Simultaneous records of urine and renal blood flows and optical records of blood pressure were made on a group of dogs under pentobarbital sodium anesthesia and on another group under chloralosan anesthesia. It was observed that pitressin, subcutaneously administered, caused no changes in urine or renal blood flows or in the blood pressure. Intramuscular injections produced variable but slight changes; but, when given intravenously, pitressin always produced transient anuria followed by oliguria for several minutes and a marked decrease in renal blood flow, from which recovery usually was not complete in half an hour, and sometimes not even in one hour. Intravenous injections of pitressin produced a sudden but transient rise in blood pressure, followed occasionally by a transient fall, and then by a gradual prolonged but moderate rise which lasted for approximately a quarter hour.

A series of observations on renal blood flow was performed on a group of trained dogs. The results under influence of pitressin injected subcutaneously, intramuscularly, or intravenously were practically the same as in the anesthetized groups. However, it was observed that the decrease in renal blood flow after intravenous administration of pitressin was more marked and usually lasted longer in trained dogs than in anesthetized dogs.

CONCLUSIONS

The data presented justify the conclusion that the same preparation of pitressin administered under identical conditions into the same animal produces different effects on the renal blood flow, depending primarily on the route of administration. Subcutaneously administered, it produces very slight, if any, change in the blood flow. Intramuscular injections give variable results, pos-

sibly dependent on the vascularity and activity of the area involved and its influence on the rate of absorption. Intravenously administered pitressin invariably decreases the blood flow, and caution regarding its intravenous use may aid in the prevention of untoward effects.

REFERENCES

1. Adolph, E. F.: Control of Urine Formation in the Frog by the Renal Circulation, Am. J. Physiol. **117**: 366, 1936.
2. Adolph, E. F.: How Pituitrin Inhibits Urine Formation, Am. J. Physiol. **116**: 1, 1936.
3. Biasotti, A.: Influence de l'extract d'hypophyse sur l'imbibition des tissus, Compt. rend. Soc. de biol. **88**: 361, 1923.
4. Brunn, Fritz: Beitrag zur Kenntnis der Wirkung von Hypophysenextrakten auf den Wasserhaushalt des Frosches, Ztschr. f. d. ges. exper. Med. **25**: 170, 1921.
5. Rey, P.: L'action des extraits post-hypophysaires sur les échanges d'eau chez Rana temporaria, Compt. rend. Soc. de biol. **118**: 949, 1935.
6. Collin, R., and Drouet, P. L.: Extrait post-hypophysaire et variations pondérales chez la Grenouille, Compt. rend. Soc. de biol. **110**: 1153, 1932.
7. Burgess, W. W., Harvey, A. M., and Marshall, E. K., Jr.: The Site of the Antidiuretic Action of Pituitary Extract, J. Pharmacol. & Exper. Therap. **49**: 237, 1933.
8. Steggerda, F. R.: The Relation of Pitressin to Water Interchange in Frogs, Am. J. Physiol. **98**: 255, 1931.
9. Richards, A. N., and Schmidt, C. F.: A Description of the Glomerular Circulation in the Frog's Kidney and Observations Concerning the Action of Adrenalin and Various Other Substances Upon It, Am. J. Physiol. **71**: 178, 1924.
10. Knisely, M. H.: A Method of Illuminating Living Structures for Microscopic Study, Anat. Rec. **64**: 499, 1936.
11. Knisely, M. H.: An Improved Fused Quartz Living Tissue Illuminator, Anat. Rec. **71**: 503, 1938.
12. Stead, E. A., Jr., Kunkel, Paul, and Weiss, Soma: Effect of Pitressin in Circulatory Collapse Induced by Sodium Nitrate, J. Clin. Investigation **18**: 673, 1939.
13. Geiling, E. M. K., Herrick, J. F., and Essex, H. E.: The Effect of Posterior Pituitary Preparations on the Blood Flow of the Normal Intact Dog, J. Pharmacol. & Exper. Therap. **51**: 18, 1934.
14. Grindlay, J. H., Herrick, J. F., and Mann, F. C.: Measurement of the Blood Flow of the Spleen, Am. J. Physiol. **127**: 106, 1939.
15. Essex, H. E., Wegria, R., Herrick, J. F., and Mann, F. C.: The Effect of Certain Drugs on the Coronary Blood Flow of the Trained Dog, Am. Heart J. **19**: 554, 1940.
16. Handovsky, H., and Samaan, Adli: Observations on the Renal Circulation and Secretion in the Dog, With Special Reference to the Effect of Pituitary (Posterior Lobe) Extract, J. Physiol. **89**: 14, 1937.
17. Janssen, S., and Rein, H.: Über die Zirculation und Wärmebildung der Niere unter Einfluss von Giften, Verhandl. d. deutsch. pharmakol. Gesellsch. In Arch. f. exper. Path. u. Pharmakol. **128**: 107, 1928.
18. Walker, A. M., Schmidt, C. F., Elsom, K. A., and Johnston, C. G.: Renal Blood Flow of Unanesthetized Rabbits and Dogs in Diuresis and Antidiuresis, Am. J. Physiol. **118**: 95, 1937.
19. Underhill, F. P., and Pack, G. T.: The Influence of Various Diuretics on the Concentration of the Blood, Am. J. Physiol. **66**: 520, 1923.
20. Verney, E. B.: The Secretion of Pituitrin in Mammals, as Shown by Perfusion of the Isolated Kidney of the Dog, Proc. Roy. Soc., London, s.B. **99**: 487, 1926.
21. Richards, A. N., and Plant, O. H.: The Action of Minute Doses of Adrenalin and Pituitrin on the Kidney, Am. J. Physiol. **59**: 191, 1922.
22. Barcroft, J., and Brodie, T. G.: The Gaseous Metabolism of the Kidney, J. Physiol. 32: 18, 1904.
23. Baldes, E. J., Herrick, J. F., and Essex, H. E.: A Modification of the Thermostromuhr Method of Measuring Flow of Blood, Proc. Soc. Exper. Biol. & Med. **30**: 1109, 1933.
24. Baldes, E. J., and Herrick, J. F. (introduced by F. C. Mann): A Thermostromuhr With Direct Current Heater, Proc. Soc. Exper. Biol. & Med. **37**: 432, 1937.

SILICON IN NONSILICOTIC LUNGS: ITS RELATION TO APICAL SCARS AND TO NODULES*

GEORGE J. MCHEFFEY, M.D., ROCHESTER, MINN.

IT HAS been accepted generally that the fibrotic scarring observed at the apices of lungs is of tuberculous origin. This view has been held until recently, when it was suggested that carbon pigment plays a part in the development of such scars. Still more recently, work has been done to demonstrate the relationship between the accumulation of silicious dust in the lungs and the presence of scars showing no evidence of healed tuberculosis.

ETIOLOGIC CONCEPTION OF APICAL SCARS

Tuberculosis.—In 1900 Naegeli[1] reported a study of the incidence of tuberculosis in 500 cases in which necropsy was performed. Apical scars were present in the lungs in 90 per cent of the cases in which the patients had lived in cities and had died of causes other than tuberculosis. He, therefore, suggested that the scars were of tuberculous origin. His view has been supported by many other workers. Aschoff,[2] in particular, stated that 90 per cent of adults have apical scars and affirmed that all of these are of tuberculous origin. Few investigators have put forth a satisfactory explanation as to why this scarring occurs. Opie[3] expressed the opinion that the lesion is a secondary type of infection derived from an exogenous source. He found apical scars in one of five or six adults in the later decades of life. He also found that many lesions were active and that the primary complex was calcified. Consequently, he expressed the opinion that this lesion is a secondary type of infection. Van Zwaluwenburg and Grabfield[4] in 1921 were convinced that this lesion is closely associated with a previous tuberculous infection of the tonsils or cervical lymph nodes. Grober[5] injected India ink into the pharynx of dogs. The dogs were killed and the lymphatics of the neck and apical regions were dissected to determine the dissemination of the ink. He found that the ink had been carried down the cervical lymphatics directly into the pleurae of the apices of the lungs. He concluded that apical scarring is brought about in this manner and that this is possibly the route of infection in many cases of pulmonary tuberculosis. Baldwin, and co-workers[6] of the Trudeau Foundation, expressed the opinion that the infection reaches the apex through the blood stream by way of the pulmonary artery. They subscribed to the hypothesis that the hilus node in the active stages of the primary infection is unable to hold all the organisms of tuberculosis that lodge there, and consequently the organisms are thrown off into the thoracic duct and thus into the pulmonary artery.

*From the Mayo Foundation, the Mayo Clinic, Rochester.

Abstract of thesis submitted to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Pathology.

Received for publication, June 9, 1941.

Dust.—While relatively little work has been done concerning the possibility of inhaled dusts as an etiologic agent for apical scars, what has been done seems to indicate a silicotic or dust origin for these scars contrary to the view of others that they are invariably of tuberculous origin. The work does not rule out, however, other inflammatory processes. Histologic examination of the lungs often shows an excess of anthracotic deposits associated with various degrees of fibrosis; and since apical scars are frequently associated with heavy anthracotic deposits, some workers have suggested that carbon pigment may play a part in the development of the scars, although at the same time they feel that the lesions are tuberculous in origin. This view has been expressed by Sato.[7] Davson and Susman[8] in 1937, studying micro-incinerated sections, concluded that the development of apical scars is secondary to the accumulation of silicious matter at the apex of the lungs. Also they showed that there is a definite relationship between silicious dust accumulating in the upper part of the lung and the scars that did not show evidence of tuberculosis.

MATERIAL AND METHODS

Micro-incineration.—Without going into the details of the technique of micro-incineration, let it suffice to say that it is a histologic method to demonstrate the presence and distribution of some of the various mineral components in tissues. The method is based upon the principle that, when a section of tissue is subjected to a temperature of 600° C., all organic material is destroyed, leaving the inorganic ashes. In these ashes are found the heat-resistant minerals, and in particular in the case of lung tissues, the deposits of the silicon compounds. The method is of value in determining the relative quantity of silicious matter in a given section of lung tissue and is of particular value in differentiating the silicotic nodule from the tuberculous nodule, as shown by Policard and Doubrow,[9] and Irwin[10] (Figs. 1, 2, 3, and 4). In the silicotic nodule the silicious ash is distributed rather uniformly throughout the entire nodule, while in the tuberculous nodule the silicious material is limited to the periphery of the nodule in the region corresponding to the capsule. Irwin inferred that if the ash pattern of silicosis is found, it represents a silicotic nodule, even if the corresponding area in the stained section has degenerated beyond recognition; and if such an area does not contain any particles of ash or only a few scattered ones, it is an area of nonsilicious fibrosis.

Method of Procedure for This Study.—The lungs in 200 patients in whom necropsy was performed at the Mayo Clinic from Sept. 1, 1938, to Dec. 31, 1938, inclusive, were examined. On gross inspection, the degree of anthracosis and the grade of apical scarring were noted. Both findings were recorded on a basis of 1 to 4. In addition, the presence or absence of evidence of healed tuberculosis in the lung or hilus lymph nodes was observed. The lungs that did not show evidence of healed tuberculosis were examined roentgenographically by Squire[11] for calcium deposits which were later recovered by him. Cases in which calcium deposits were found in the lungs were added to those cases of healed tuberculosis already ascertained by visual inspection. Finally, the age, sex, occupation, and residence of the deceased were recorded. The great majority of these 200 persons were adults more than 20 years of age,

while the minority consisted of younger persons, including a few children. Also, the lungs and portions of lungs in several known cases of silicosis were examined. A similar examination was made of the lungs of a few newborn infants. The latter two sets of lungs, those from persons who had had silicosis

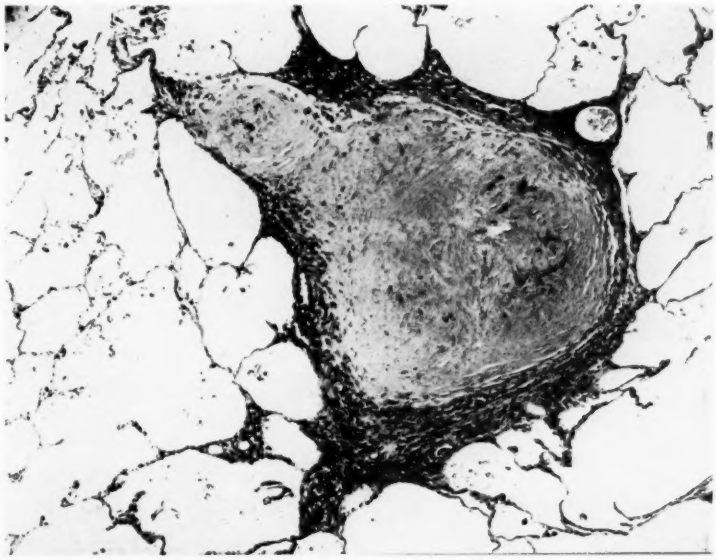

Fig. 1.—Silicotic nodule in a known case of silicotuberculosis (hematoxylin and eosin, ×42).

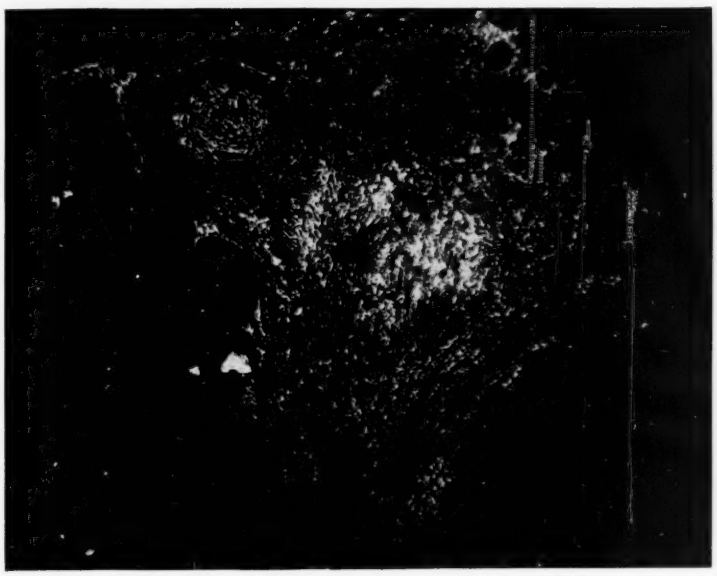

Fig. 2.—Consecutive section to Fig. 1, incinerated, showing ash pattern of a silicotic nodule (dark field, ×42).

and those from infants, were examined for the purpose of comparison and control, but neither of these sets was included in the 200 afore-mentioned cases, none of which revealed any evidence of clinical silicosis during life.

The lungs were sectioned longitudinally at intervals of ½ inch (1.3 cm.) to determine whether or not there was any gross evidence of silicosis, such as the presence of a nodule in the lung or hilus nodes, the presence of "egg shell"

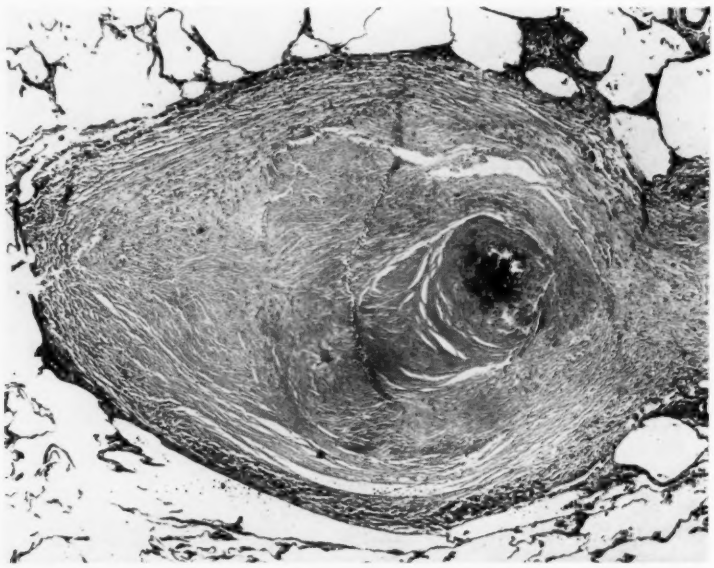

Fig. 3.—Tuberculous nodule situated near the silicotic nodule shown in Fig. 1 (hematoxylin and eosin, ×34).

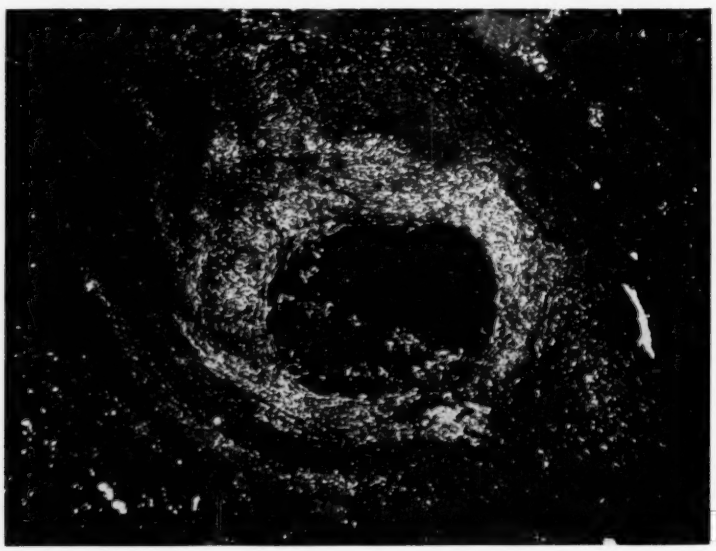

Fig. 4.—Consecutive section to Fig. 3, incinerated, showing ash pattern of a tuberculous nodule (dark field, ×34).

calcification in the hilus nodes, or the presence of suspicious regions of fibrosis. If any such lesions were discovered, blocks of tissue were made of the region involved and were placed in a fixative of 10 per cent formalin. Then routinely one block of tissue was cut from the apical scar or from the apex in those lungs

not revealing a scar, one block from the upper lobe near the scar but not including it, one block from the lower lobe, and one of a hilus lymph node that did not contain calcareous material. These blocks, after fixing, were embedded in paraffin in the usual manner, and serial sections were cut of a thickness of 5 microns. Three sections were cut, one for staining with hematoxylin and eosin, and two unstained sections for micro-incineration. After incineration the sections were treated with concentrated hydrochloric acid so as to remove the inorganic ash, and then were examined with dark-field illumination. By comparison with the consecutive stained sections, an exact localization of any deposits of silicious ash was obtained. Furthermore, since all sections were of equal thickness, the amount of silicious ash was compared by visual estimation.

For the purpose of estimation of the amount of silica in any given section, four grades were recognized: grade 1, slight amount of ash deposits about the blood vessels or bronchioles; grade 2, moderate amount of ash deposits about the blood vessels or bronchioles associated with a slight amount of diffuse scatterings of ash throughout the parenchyma; grade 3, large collections of ash deposits about vessels and bronchioles associated with a uniform scattering of ash throughout the parenchyma; and grade 4, large collections of local deposits associated with large deposits throughout the parenchyma. In the case of silica grade 4, the amount corresponded to that found in sections from the fibrotic portions of silicotic lungs.

RESULTS

The apical scar lesion occurred at the apex of either one or both lungs and was characterized by a firm, grayish-white, opaque, "scarred" region with variable degrees of puckering of the visceral pleura. In some cases the scarring was more extensive and formed a cap. A few of these caps felt on palpation as if they were constructed of cartilage. Sections of the region had in some cases a dark anthracotic fibrosis in the subjacent lung substance without any evidence of caseation or calcification. A few scars revealed gross evidence of calcification. In some cases the parenchyma of the lung directly beneath the scar contained an emphysematous cavity or multiple smaller ones.

By histologic examination there was fibrous thickening of varying depth, and the subjacent lung parenchyma revealed a greater or lesser degree of fibrosis. The fibrous tissue when stained with hematoxylin and eosin usually exhibited a bluish pink to pink color of the connective tissue fibers. In many cases the connective tissue had become hyalinized. The extent of the fibrotic regions varied; in younger people the lesions were smaller, while in the older age groups the more massive lesions usually occurred. There was considerable proliferation of collagen fibers throughout the fibrotic regions in some of the lesions, some of which could be traced into the parenchyma of the adjacent lung and merged with it. The fibrotic regions contained varying amounts of carbon pigment scattered diffusely throughout the scar. In some cases numerous alveoli containing clumps of phagocytes laden with carbon occurred within the fibrotic regions. In four scars giant cells were seen.

Micro-incineration of the apical scars showed that the deposits of silica were always present with the carbon and in corresponding amounts, except for

a few cases in which the silicious matter was greater than the corresponding carbon and vice versa (Figs. 5, 6, 7, and 8). Also as far as the lungs were concerned, the silicious material was found to be more or less uniform throughout, although there was a moderate increase in the silica grade in the upper

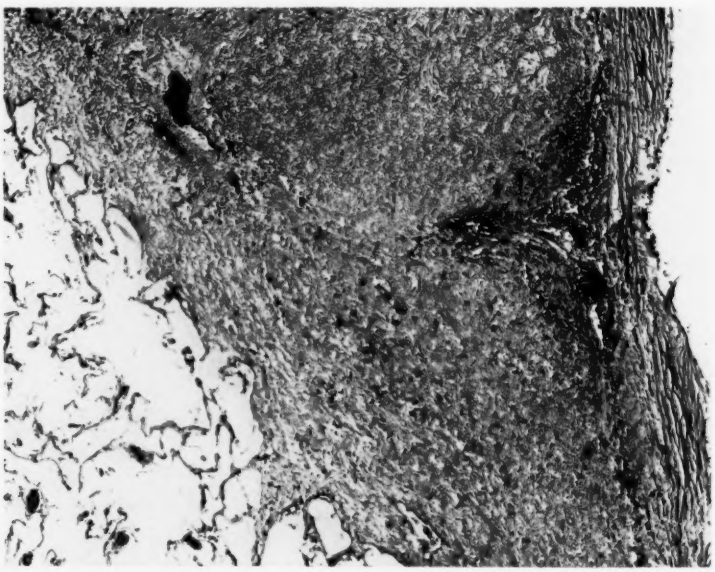

Fig. 5.—"Nonspecific" apical scar (hematoxylin and eosin, ×42).

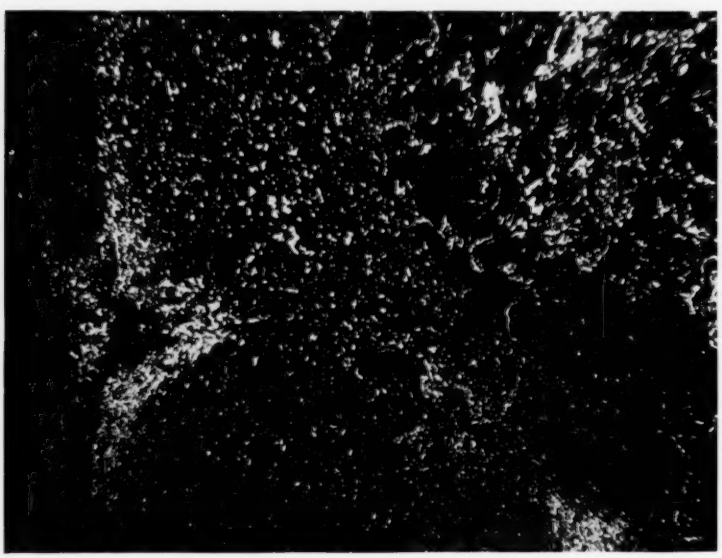

Fig. 6.—Consecutive section to Fig. 5, incinerated, showing diffuse and local collections of silicious deposits in precisely the same locations as the carbon deposits (dark field, ×42).

lobes. In a few apical scars and in many lungs and hilus lymph nodes the intensity of silica was quite comparable to that seen in microscopic sections from silicotic lungs. In these cases there were seen in the stained section areas of fibrosis characterized by circumscribed hyalinized areas which appeared to

be identical with silicotic nodules. They contained the finely distributed carbon sprinkling commonly seen in silicotic nodules. Incineration of these lesions revealed that they contained the silica ash pattern appearing very similar to those found in known silicotic nodules. In some of the better incinerated

Fig. 7.—"Specific silicotic" or dust "apical scar." Note silicotic nodule in the apical scar (hematoxylin and eosin, ×48).

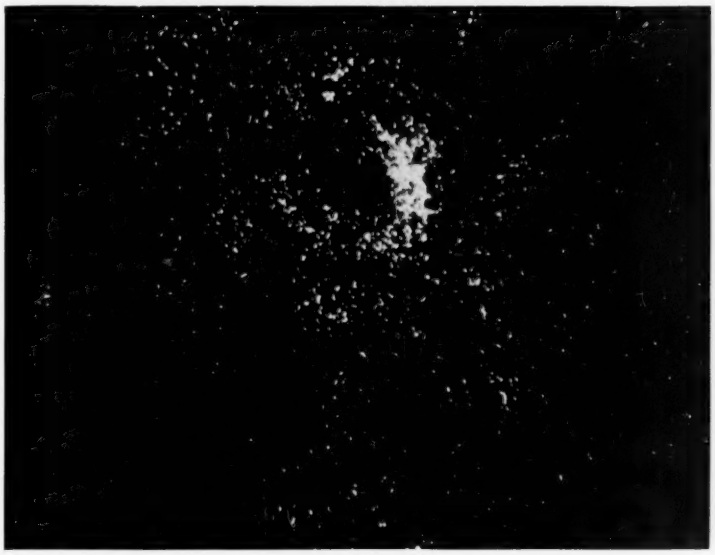

Fig. 8.—Consecutive incinerated section to Fig. 7 (dark field, ×48).

sections of apical scars there was noted the ash pattern of the scar, and in the background appeared a faint ash network which somewhat resembled the architecture of lung tissue and which merged with the ash pattern of the normal adjacent lung. This ash pattern seen in the background of the fibrotic

areas was no doubt the lung that previously had occupied the area and which was replaced by collagenous connective tissue.

The findings of this study are given in seven different tables. Table I summarizes the number of cases of apical scars in both sexes and their relation to the age of the patients and to the amount of anthracosis in their lungs. There was no anthracosis in the first decade of life; anthracosis grade 1 began in the second decade, with a maximal number of cases in the seventh decade; anthracosis grade 2 began in the third decade, with a maximum in the sixth decade; anthracosis grade 3 began in the fifth decade, with a maximum in the seventh decade; and anthracosis grade 4 occurred only in the seventh decade. Of the 133 cases of apical scars (66.5 per cent) the least number occurred in the third and ninth decades, and the greatest number in the seventh decade. Cases of apical scarring did not occur during the first two decades. Finally, it is noted that the degree of anthracosis increased with the age and the number of cases of apical scars increased with age as the grade of anthracosis increased, this being true up to the higher decades of life, at which point the number decreased.

TABLE 1

GRADE OF ANTHRACOSIS AND OCCURRENCE OF APICAL SCARS BY AGE OF PATIENTS, BOTH SEXES

AGE (YR.)	CASES	GRADE OF ANTHRACOSIS					APICAL SCARS	
		0	1	2	3	4	CASES	PER CENT
0-9	2	2	0	0	0	0	0	0.0
10-19	10	3	7	0	0	0	0	0.0
20-29	10	0	9	1	0	0	6	60.0
30-39	14	0	13	1	0	0	8	57.0
40-49	28	0	20	7	1	0	18	64.0
50-59	46	0	30	14	2	0	35	76.0
60-69	54	0	40	8	4	2	39	72.0
70-79	25	0	15	8	2	0	21	84.0
80 and more	11	0	7	3	1	0	6	54.0
Total	200	5	141	42	10	2	133	66.5

TABLE II

AVERAGE AGE OF PATIENTS AND OCCURENCE OF APICAL SCARS BY GRADE OF ANTHRACOSIS

GRADE OF ANTHRACOSIS	SEX		TOTAL CASES	AVERAGE AGE (YR.)	APICAL SCARS	
	MALE	FEMALE			CASES	PER CENT
0	2	3	5	8.6	0	00.0
1	81	60	141	54.5	89	63.1
2	35	7	42	58.5	34	81.0
3	10	0	10	65.0	8	80.0
4	2	0	2	66.5	2	100.0
Total	130	70	200		133	66.5

While Table I reveals the relationship of apical scars and anthracosis to the various age groups, a much better relation is obtained by studying Table II, which shows the relationship of anthracosis and apical scars to the average age. Here it is noted that the majority of cases are anthracosis grade 1, and that the number of cases decreases as the grade of anthracosis increases beyond grade 1, but that the average age of the patients increases. The percentage of apical scars increases with the average age and with the grade of anthracosis.

Thus far, the discussion has been concerned mostly with anthracosis. The reason for this will be obvious when Table III is studied. This table, con-

structed exactly like Table II, shows the relationship of apical scars to the average age and to the total deposits of silica in the lungs, as estimated by micro-incineration. Here it is noted that of the 200 cases studied, 157 reveal silica grade 1 in the lungs, while a decreasing number of cases reveal silica, grades 2, 3, and 4, respectively. Ten cases reveal no silica at all, and the average age of this group is 24 years. Of the cases of silica grade 1, the average age is 54.3 years. Of the grades of silica higher than 1, the average age increases for each silica grade group, except grade 4, while the total number of cases for each silica grade decreases beyond the peak of silica grade 1. As concerns the apical scars, here again it is noted that the percentage of apical scars increases with age as the total content of silica of the lungs increases. After studying Tables II and III, one is struck by the close parallelism of the gross anthracotic grade with the microscopic total silica grade with respect to the total number of cases, the average age, and the percentage of apical scars. Thus far, the study seems to verify the findings of previous workers, namely, that silica deposits occur in conjunction with anthracotic deposits, and in roughly corresponding amounts. In addition, the findings suggest that the amount of anthracosis determined by gross examination of the lungs is a fairly good index to the total amount of silicious material present in the same lungs.

TABLE III

AVERAGE AGE OF PATIENTS AND OCCURRENCE OF APICAL SCARS BY GRADE OF TOTAL SILICA IN LUNGS AS ESTIMATED BY MICRO-INCINERATION

GRADE OF TOTAL SILICA	SEX		TOTAL CASES	AVERAGE AGE (YR.)	APICAL SCARS	
	MALE	FEMALE			CASES	PER CENT
0	6	4	10	24.0	2	20.0
1	95	62	157	54.3	104	66.2
2	25	4	29	64.3	23	79.3
3	3	0	3	69.6	3	100.0
4	1	0	1	68.0	1	100.0
Total	130	70	200		133	66.5

What has been said concerns the apical scar and its relation to the silica deposits in the lungs. Now what about the relation of this lesion to the silica deposits in the scar itself, and the relation of the latter to the silica deposits in the respective lungs and hilus lymph nodes? It is noted in Table IV that as the thickness of the apical scar increases, the total content of silica in the apical scars and their respective lungs and hilus nodes tends to increase. This is much more evident in the male sex.

Of the 133 cases of apical scars, it was of interest to note that in only four cases (3.0 per cent) and possibly in only 3 cases, was there no evidence of tuberculosis in the lungs or hilus lymph nodes. In other words, in 129 cases of apical scars (97.0 per cent) there were signs of tuberculosis in either the lung or the hilus lymph nodes. Of the 4 nontuberculous patients, 2 were men and 2 were women. One patient was a 73-year-old man with a very thick apical scar and a correspondingly high silica content in the scar, lung, and hilus nodes, while the other 3 cases were in young persons (ages 24, 30, and 32 years) with correspondingly low silica determinations. While it is possible for an old man not to have evidence of healed pulmonary tuberculosis, it must be stated that this was the only case in which roentgenologic examination of the lungs for calcium deposits

was not made. Hence the previous statement that possibly in only 3 cases was there no evidence of tuberculosis in the lungs or hilus nodes is probably correct.

TABLE IV

APICAL SCARS WITH RELATION TO THEIR SILICA CONTENT

SEX	GRADE OF SCAR	CASES	TOTAL SILICA, AVERAGE GRADE ON BASIS OF 1 TO 4		
			SCAR	LUNG	HILUS NODE
Male	1	47	1.4	1.3	2.2
	2	26	1.7	1.3	2.4
	3	10	2.1	1.9	2.6
	4	5	2.0	1.2	2.2
Female	1	28	1.1	0.9	2.3
	2	12	1.6	1.3	2.3
	3	4	1.6	1.2	2.3
	4	1	1.0	0.7	2.0
Total		133			

Of the 67 cases without apical scars, there was evidence of tuberculosis in the lungs or hilus nodes in 48 (72 per cent) (Table V). The average total content of silica in the lungs and hilus nodes of the men without apical scars, but with evidence of tuberculosis, is found to be somewhat less than for the men with apical scars. As to the female sex, the corresponding silica determinations are considerably less than for those females having apical scars.

TABLE V

PATIENTS WITH NO APICAL SCARS WITH TUBERCULOSIS PRESENT IN LUNGS OR HILUS NODES

SEX	CASES	AGE, YEARS			TOTAL SILICA, AVERAGE GRADE	
		MEAN	YOUNGEST	OLDEST	LUNG	HILUS NODE
Male	36	54.5	20	97	1.2	2.2
Female	12	48.3	11	83	0.8	1.8

Table VI summarizes the remaining 19 cases, in which there were no apical scars and no evidence of tuberculosis in the lungs or hilus nodes. In general, the patients without evidence of tuberculosis were those in the lower average age groups, and the average total silica was also correspondingly low. On the other hand, the patients that did reveal tuberculosis were in the higher average age groups, and the total content of silica was higher. In nearly all instances the silica content of male patients was somewhat higher than that of female patients.

TABLE VI

PATIENTS WITH NO APICAL SCARS WITH NO TUBERCULOSIS PRESENT IN LUNGS OR HILUS NODES

SEX	CASES	AGE, YEARS			TOTAL SILICA, AVERAGE GRADE	
		MEAN	YOUNGEST	OLDEST	LUNG	HILUS NODE
Male	7	34.1	11	68	0.6	1.2
Female	12	40.8	2	76	0.7	1.1

In passing, a word might be mentioned concerning the general incidence of healed tuberculosis in the 200 cases studied. In 96 cases evidences of healed tuberculosis were found in the lungs by gross dissection. Squire,[11] by post-mortem roentgenologic examination of the lungs, found 28 more cases. Two

cases were found by microscopic examination. This makes a total of 126 cases in which evidences of healed tuberculosis were found in the lungs. In the hilus nodes 127 cases were found by gross dissection. Squire found 38 more cases by roentgenologic examination, making a total of 165 cases in which evidences of healed tuberculosis were found in the hilus nodes. Thus, according to this survey of 200 cases, the incidence of healed tuberculosis is 177 cases, or 88.5 per cent.

"Silicotic" or "Dust" Nodules in Apical Scars.—It was previously stated that lesions were found in the lungs or hilus nodes that resembled silicotic nodules and which, upon micro-incineration, revealed the ash patterns of silicotic nodules. In 7 of 133 cases of apical scars, there was an ash pattern comparable to that of known silicotic nodules (Figs. 7 and 8). Thus, the incidence of apical scars that can be said to be "specific silicotic" or "dust scars" is 5.3 per cent. Table VII summarizes these cases. It is noted that in 5 cases the patients were men and in 2 cases they were women, the average age for all being 69 years. The average grade of the apical scars is 2+, and the total silica grade in each scar and hilus node is high, while the silica content of the lung varies. However, note that there is a fairly close correlation between the silica content of the apical scars and the silica content of the hilus nodes.

TABLE VII

PATIENTS WITH SILICOTIC NODULES IN APICAL SCARS

SEX	AGE (YR.)	OCCUPATION	GRADE OF APICAL SCAR	TOTAL SILICA, GRADE		
				SCAR	LUNG	NODE
M	67	Street car conductor	3	4.0	3.0	4.0
M	55	Civil engineer	2+	2.0	1.0	3.0
M	77	Farmer	4	3.0	3.0	4.0
M	71	Auto trimmer	1	3.0	2.0	3.0
M	73	Miller	2	3.0	1.5	3.0
F	55	Housewife	2	3.0	1.0	4.0
F	85	Housewife	2	3.0	1.5	4.0
Average	69		2+	3.0	1.9	3.6

"Specific Tuberculous" Apical Scars.—In 4 of the 133 cases of apical scars, there were giant cells, in one case there were tubercles, and in 12 cases there were calcium deposits in the scars themselves. This makes a total of 17 cases of "tuberculous" apical scars (12.8 per cent). In these 17 cases the average age of the patients was 60 years, and the average apical scarring was grade 2+. However, the average silica content of the scar, lung, and hilus node was 1.5, 1.2, and 2.3, respectively, an amount considerably lower than the corresponding average silica content in the "specific silicotic" apical scars.

Since 5.3 per cent of the apical scars are "silicotic" and 12.8 per cent are "tuberculous," the total number of "specific apical scars" is 18.1 per cent. What of the apical scars making up the remaining 81.9 per cent? These apical scars are the "nonspecific scars," characterized by a more or less hyalinized fibrosis of variable thickness, all of which contain some silicious deposits, and all of which, with the exception of 3 or possibly 4 cases, are associated with healed tuberculosis, either in the lungs or hilus lymph nodes or both.

Incidence of the Silicotic Nodule in the Nonsilicotic Lung.—By microincineration the ash pattern of the silicotic nodule was found to be present in 21 cases.

Seventeen patients were male and 4 were female with an average age of 68.4 years. The silicotic ash pattern was found in 7 apical scars, in 5 lungs, and in 17 hilus nodes. The finding of the lesion in both the apical scar and the hilus node in the same subject occurred in 2 cases, while the presence of the lesion in the apical scar, in the lung, and in the hilus node in the same subject occurred in 3 cases. The local silica value for all these lesions was high, while the total silica content was relatively high in the respective lungs and hilus nodes. Even the average silica content for all 21 cases was seen to be higher than that found in the great majority of the 200 cases studied. Therefore, on the basis of this study of 200 cases, the incidence of the silicotic nodule in the nonsilicotic lung is 10.5 per cent. In general, the nodules were considerably smaller than the silicotic nodules seen in advanced cases of silicosis. Of all the cases studied, there were none in which the occupation of the patient was predisposed to silicosis; yet there were 21 cases in all of which one or more silicotic nodules were observed. There was little difficulty in distinguishing between healed tuberculous nodules and the nodules due to silica deposits when the method of microincineration was employed. In a few lungs not included in the series of 21 cases, early stages of formation of silicotic nodules were seen in the fibrous capsule of healed tuberculous nodules. It is known that carbon tends to collect around the fibrous walls of such lesions, and in these cases enough silicious material was present, as shown by micro-incineration, to produce the early stages of formation of silicotic nodules.

Considering all cases examined, this study demonstrates that silica deposits are present in normal lungs along with carbon deposits and are coextensive with them. Moreover, the deposits occur in localized regions in quantities comparable with those obtained in cases of diffuse silicosis. Therefore, all persons are exposed to a common source of dust particles. According to Policard and Doubrow, the source might be the smoke from domestic and industrial furnaces, since a high percentage of coal ash is silica. Jephcott[12] quoted figures published by the Chicago Board of Health in 1931, estimating that 134 tons of silicious matter fall per month per square mile. This suggests that all city dwellers during their lifetime inhale appreciable amounts of silica. It is also noteworthy that Campbell[13] found 44 per cent of silica in the dust from tarred roads.

COMMENT

By comparing the silica content of the upper lobes with that of the lower lobes it was found that in nearly every case the intensity of carbon and silica accumulations was less in the lower than in the upper lobes. This tendency might be explained by the relative immobility of the apex, as compared with the greater mobility of the lower lobe, so that particles once inhaled tend to lodge in the apical region, while the better ventilation of the rest of the lung tends to favor the removal either by the bronchi or by the lymphatic drainage. Thus, since silicious material tends to collect in the apical region, it is probable that the tendency for scars to occur at the apex is linked to some degree with such dust deposits. This is stated with reserve, because it is quite well known that accumulations of silica occur in regions of fibrosis, regardless of cause. Although the average total silica determined in all the "specific tuberculous" apical scars

is lower than that of the "specific silicotic" scars, there are individual instances in which the silica value is comparable to the dust accumulations found in other apical scars.

CONCLUSIONS

1. Anthracotic and silicious dust deposits in the lung tissue increase with age, and the grade of anthracosis in a given lung is a rough index to the relative amount of silica deposits present in the same lung, since silica deposits occur in conjunction with anthracotic deposits and in corresponding amounts.

2. The average total content of silica in the lungs of the male is higher than that of the female.

3. The percentage of cases of apical scars increases with age and with the grade of the total silica content of the lung.

4. Of the cases of apical scars 97.0 per cent reveal evidence of healed tuberculosis elsewhere in the lung or hilus lymph nodes.

5. The average total content of silica in lungs possessing apical scars is higher than in those lungs that do not have apical scars.

6. On the basis of 200 cases the incidence of healed tuberculosis in the lungs is 88.5 per cent.

7. On the basis of 133 cases of apical scars the incidence of "specific silicotic" apical scars is 5.3 per cent, and the silica content in these specific cases is relatively high.

8. On the same basis the incidence of the "specific tuberculous" apical scars is 12.8 per cent, and the average silica content in these cases is considerably lower as compared to the "specific silicotic" scars.

9. The incidence of the silicotic nodule in the nonsilicotic lung is 10.5 per cent.

REFERENCES

1. Naegeli, Otto: Ueber Häufigkeit, Localization und Ausheilung der Tuberculose, Virchows Arch. f. path. Anat. **160**: 426, 1900.
2. Aschoff, L.: Über den phthisischen Reinfekt der Lungen, Klin. Wchnschr. **8**: 1, 1929.
3. Opie, E. L.: Pathological Evidence of First Infection in Association With Active Pulmonary Tuberculosis, Am. Rev. Tubere. **10**: 249, 1924.
4. Van Zwaluwenburg, J. G., and Grabfield, G. P.: The Tonsillar Route of Infection in Pulmonary Tuberculosis, Am. Rev. Tubere. **5**: 57, 1921.
5. Grober, J. A.: Die Infectionswege der Pleura, Deutsches Arch. f. klin. Med. **68**: 296, 1900.
6. Baldwin, E. R., Petroff, S. A., and Gardner, L. S.: Tuberculosis: Bacteriology, Pathology and Laboratory Diagnosis With Sections on Immunology, Epidemiology, Prophylaxis and Experimental Therapy. The Trudeau Foundation Studies. Philadelphia, 1927, Lea and Febiger, p. 162.
7. Sato, S.: Quoted by Davson, J., and Susman, A.
8. Davson, J., and Susman, A.: Apical Scars and Their Relationship to Silicious Dust Accumulation in Non-silicotic Lungs, J. Path. & Bact. 45: 597, 1937.
9. Policard, A., and Doubrow, S.: Etude histochemique de l'anthracose pulmonaire; déductions pathogéniques, Presse méd. **2**: 905, 1929.
10. Irwin, D. A.: The Histological Demonstration of Silicious Material by Microincineration, Canad. M. A. J. **31**: 135, 1934.
11. Squire, E. W.: Personal communication to the author.
12. Jephcott, C. M.: Some Aspects of the Chemistry of Silicosis, Canad. Pub. Health J. **26**: 606, 1935.
13. Campbell, J. A.: The Effects of Road Dust "Freed" From Tar Products Upon the Incidence of Primary Lung-tumours of Mice, Brit. J. Exper. Path. **18**: 215, 1937.

FOOD REMNANTS AS A CAUSE OF CONFUSION IN THE DIAGNOSIS OF INTESTINAL PARASITES*

NADENE DENISON, M.S., NEW ORLEANS, LA.

INTRODUCTION

ONE of the everyday problems of the diagnostician in the clinical parasitology laboratory is the differentiation of helminth eggs and protozoan cysts of pathogenic parasites of the intestine, not only from nonpathogenic species and free living organisms, but from various remnants of foods present in feces. The latter, due to their size and general appearance, are often confused with parasites. The object of this study was to find out experimentally what foods are responsible for some of the remains so frequently confused with intestinal parasites.

As early as 1717 van Leeuwenhoek, in examining human exudates, was able to demonstrate that the remains of foods eaten could be recognized in fecal material. The studies initiated by the idea that diseases could be diagnosed by crystals in excreta and the interest aroused in the physiology of digestion in the late nineteenth century led to several works on the composition of the feces. An early worker in this field, Rawitz (1845), classified the residual particles in feces as animal residue, plant residue, single cells, and foreign bodies, but his meaning, particularly in regard to the identity of the "single cells" and "foreign bodies," is not clear. Chiefly concerned with the normal and faulty digestion of foods, much of the work since 1899 has been based upon an extensive monograph by H. L. Q. van Ledden Hülsebosch, who discussed the macroscopic and microscopic structures of numerous plant foods. An excellent summary of the knowledge of fecal constituents was given in 1914 by Cammidge, who recognized the difficulties of gross parasitologic diagnosis, especially of the smaller nematodes and tapeworm proglottids, with which numerous fibers are easily confused upon macroscopic examination. Some anomalies found in microscopic fecal examinations were reported by Faust from China in 1924. Structures which he listed as confusing to the parasitologist were physical artifacts, contaminations, partially digested animal and plant cells, animals introduced by accident, harmless coprozoites, and body cells or tissues produced by noninfective agencies.

EXPERIMENTAL DATA

In order to secure a point of departure for the study of the origin of some of the confusing remains, it was deemed advisable to find out which of the more common foods were most likely to leave particulate remains resembling

*From the Department of Tropical Medicine, Tulane University of Louisiana, New Orleans, La.

Received for publication, June 16, 1941.

intestinal parasites in human feces. These undigested or partially undigested food remnants found in feces are difficult, if not at times almost impossible, of identification. However, by becoming acquainted with the structural elements of any given food before it was eaten, it was possible to determine its more characteristic residues. To do this a basic nonresidue diet was used, and for the checking of each individual food studied, a controlled diet was taken. For the remainder of the work hospital patients were used; these patients were on identical diets and their digestion was for all purposes apparently normal.

The basic diet consisted of strained vegetable and fruit juices, ground cooked meat, mashed Irish potatoes, milk, coffee, and white bread. Since cellular structure was absent in the liquids, was destroyed by cooking the bread and potatoes, or was easily recognized, as in the case of the meat, this diet served the purpose well. A small quantity of each food to be studied was obtained in the form in which it was to be served, and was teased apart in saline to separate the various structural elements. Drawings were made of these cells at an average magnification of ×540. Stools passed twelve to fifteen hours after the food was eaten were examined for remains of fibers and other structures. These remains were studied, and sketches were made of their characteristic appearance. In both cases the material was studied unstained as well as stained with D'Antoni's iodine.

Animal Foods.—Such animal products as milk, cheese, custards, gelatin, and cooked eggs may be classified as nonresidue foods, except when eaten in excessively large amounts, such as milk in the dietary of infants. Of the animal tissues studied, as liver and muscle, only the muscle retained a close resemblance to its original structure, and fecal remnants of muscle fibers could be recognized as such without difficulty. Of the sea foods, crab meat maintained the characteristic structure of arthropod muscle fibers, but oysters and most fish left little residue.

Plant Foods.—Undigested plant foods in the form of single cells left the most confusing residues and those most likely to be mistaken for the eggs or cysts of parasites. A variety of common vegetable foods was studied, including representative grains eaten as cereals or in breads, fruits both fresh and dried, leafy vegetables, roots, and some leguminous seeds.

Fragments of whole vegetable tissues were not difficult to recognize as such, for their characteristic cellular arrangements obviate confusion with animal parasites. Bits of the husk of oats and whole corn were often observed to be almost undigested, as well as large pieces of leaf or epidermal tissues and aggregates of fibers from fibrovascular bundles.

Single epidermal cells separated from their tissue did, in a few cases, resemble eggs or cysts, but their heavy cellulose walls and complete lack of internal organization were diagnostic of their plant origin. More confusing were epidermal gland cells or epidermal hairs which, separated from the remainder of the tissue, respectively, resembled tapeworm eggs and small nematode larvae. Palisade cells, particularly from beans or peas, serve as diagnostic landmarks not only for the type of vegetable but also for the particular kinds of legumes, since they are characteristic for each plant, but they are easily differentiated from parasites on the basis of their thick walls and lack of internal structure.

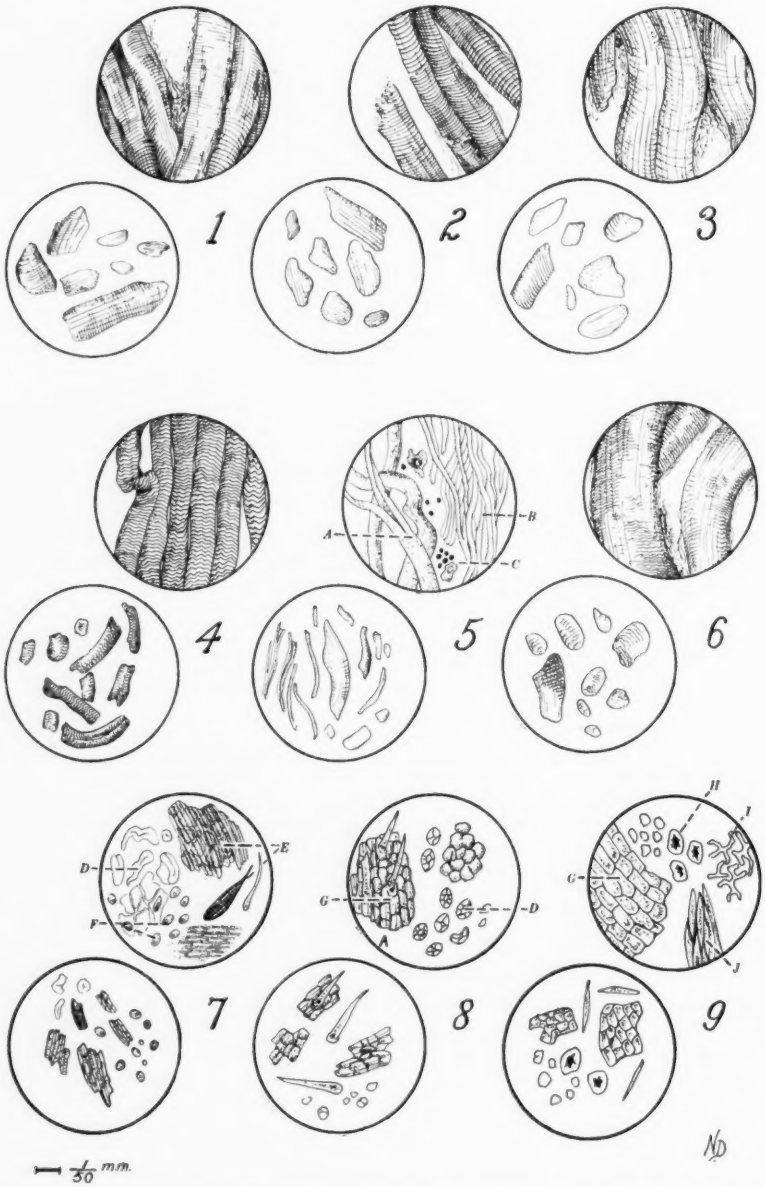

Plate I.—Plates I, II and III represent the characteristic structures of each prepared food, together with their remnants commonly found in feces. The whole foods are figured upward and to the right, remnants are below and to the left of each figure number. *1*, Beef muscle; *2*, corned beef muscle; *3*, pork muscle; *4*, crab meat; *5*, oyster; *6*, fish muscle; *7*, wheat; *8*, oats; *9*, whole kernel corn.

A, Muscle fibers; *B*, elastic fibers; *C*, parenchyma cells; *D*, starch; *E*, fragments of husk; *F*, aleurone grains; *G*, epidermal cells; *H*, stone cells; *I*, tube cells; *J*, bast fibers; *K*, fibrovascular bundles; *L*, "skin" of seed; *M*, epidermis with gland cell (*g*) and stomata (*s*, *s*); *N*, palisade cells.

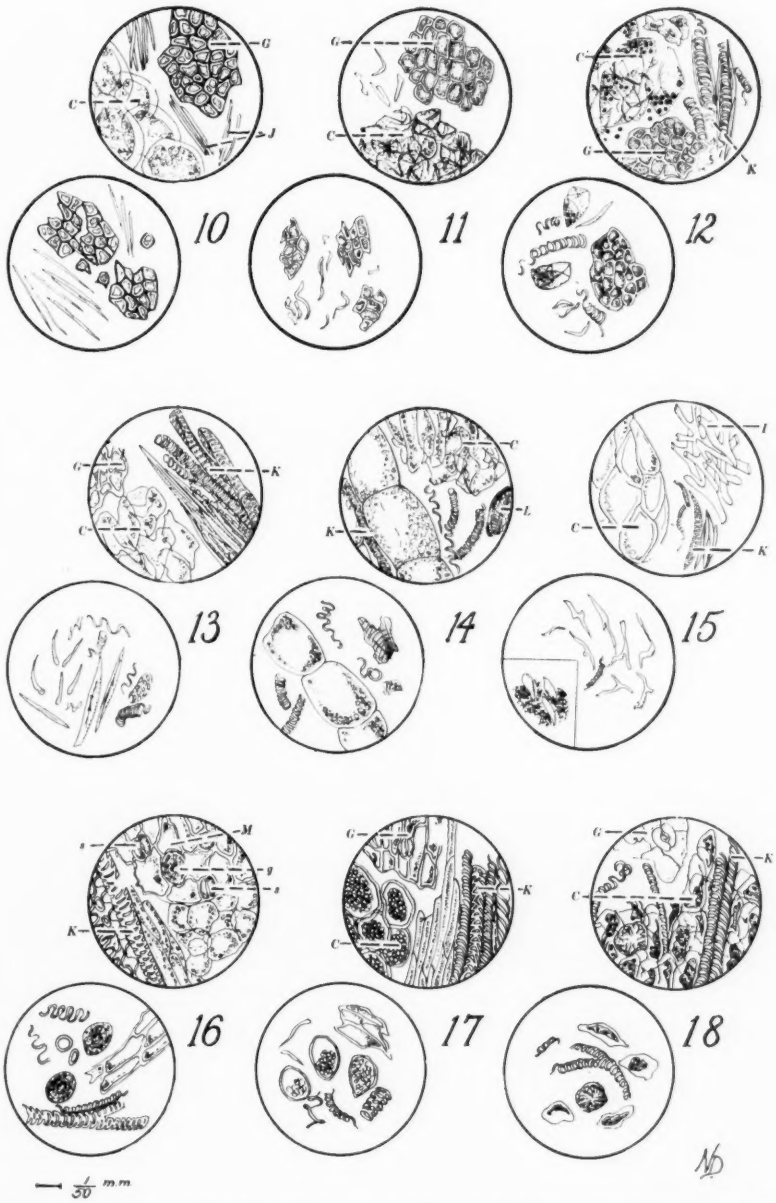

Plate II.—*10*, Apple; *11*, raisin; *12*, prune; *13*, peach; *14*, banana; *15*, orange; *16*, lettuce; *17*, mustard greens; *18*, spinach. (*See* Plate I legend for explanation of letters.)

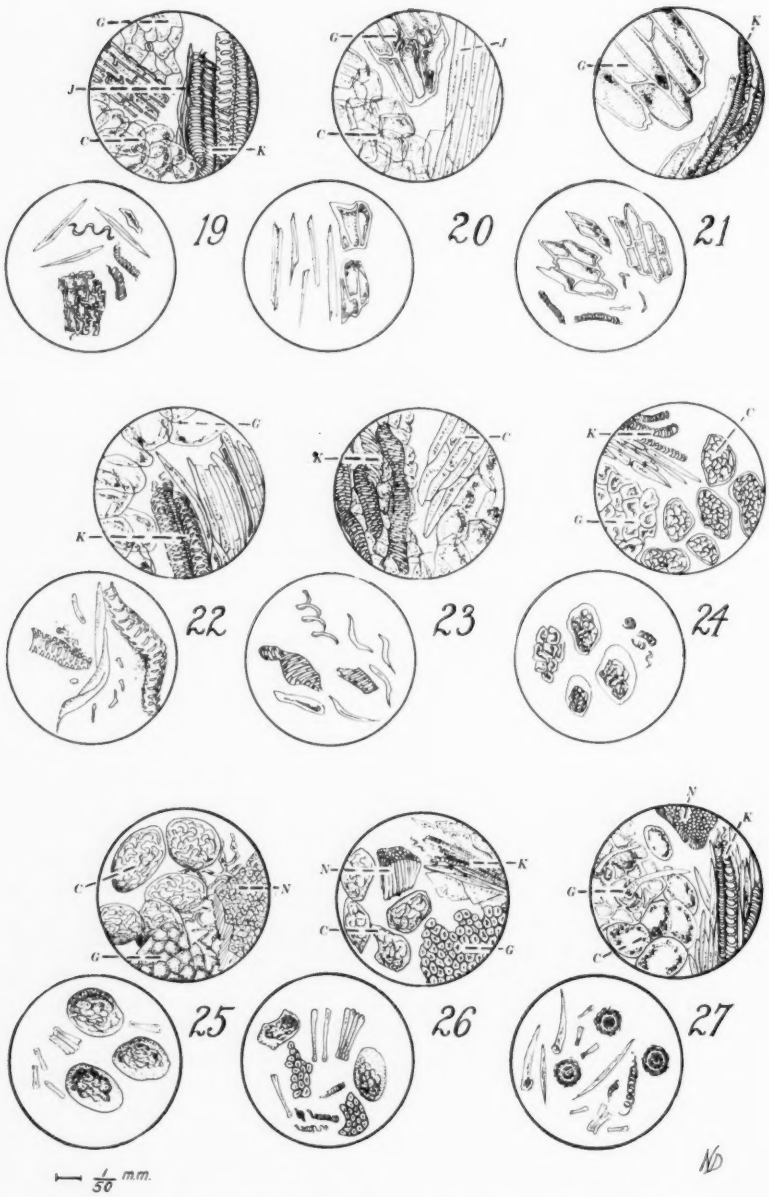

Plate III.—*19*, Cabbage; *20*, celery; *21*, onion; *22*, beet; *23*, carrot; *24*, sweet potato; *25*, pea; *26*, lima bean; *27*, string bean. (*See* Plate I legend for explanation of letters.)

The smaller particles from fibrovascular bundles are the characteristic lignated spirals and tubes, even fragments of which could be rarely ever confused with animal cells. Bast fibers, on the other hand, elongate lignated elements of the fibrovascular bundles, frequently simulate nematode larvae, the criterion of differentiation here again being the very thick walls and lack of internal structure in the plant cell. Stone cells, particularly of fruits, appeared frequently in feces; their shape and size at times closely resembled the eggs and cysts of parasites. Parenchyma cells were the most common of all cell types found singly, and, because of their shape and the presence of cytoplasm, might easily be confused with parasites. Because of the protection from digestion afforded by their cellulose walls, the remaining abundant cytoplasm allows the parenchyma cells of leafy vegetables, roots, and legumes to resemble helminth eggs, except for the lack of cellular organization or embryonic membranes. Parenchyma cells of bananas which frequently remain attached end-to-end have the appearance of minute tapeworm segments, but they lack internal organization or cuticular structures.

Starch granules observed in this study were for the most part destroyed by cooking; hence they were infrequently characteristic either in their staining reaction to iodine or in their structural appearance. Aleurone granules from grains often appeared much like minute protozoan cysts and might be very confusing unless closely studied.

Plates I, II, and III portray the characteristic appearance of the individual food elements studied both before and after digestion.

DISCUSSION

Foods ready to be served and the remnants occurring in feces have been studied from individuals whose digestive processes were apparently normal. Animal foods left no detritus that might cause any confusion in parasitologic diagnosis, but vegetables present a wide variety of remains, especially single-celled elements which closely simulate parasitic cysts or eggs. Fairly large, heavy-walled cells with shrunken cytoplasm, occurring as fecal remains and simulating helminth eggs, are usually derived from potatoes, leguminous seeds, such as peas and beans, or occasionally from various greens. Smaller cells and aleurone granules may simulate minute protozoan cysts.

It is not particularly difficult to determine into which general groups of plant foods fecal remnants may be classified, for as a rule some tissue fragments as well as single cells will occur, and a tentative diagnosis can be made from a consideration of the collective residues as to the foods eaten. Since such tissue fragments, because of their group organization, are not confusing, it is the undigested cells found in feces that are difficult to diagnose. However, their structure or their lack of structural organization should obviate errors in parasitic diagnosis, for, unlike protozoan cysts or helminth eggs, these cells are irregular in shape and size, their cytoplasm is shrunken, they rarely have nuclei, and they lack the internal order common in animals. Thus, even though a number of foods after apparently normal digestion have residues which might be confused with parasites, sufficient structures are left to prevent diagnostic mistakes.

SUMMARY

1. An experimental study was conducted to ascertain which of the more common foods leave remnants in the feces which might be confused with intestinal parasites or their products.

2. A controlled nonresidue diet was used, to which various foods were added one at a time to allow study of the characteristic remnants of each food.

3. Foods which had no residue of any importance at all were animal products, such as milk, cheese, and eggs, and sea foods, such as oysters.

4. Animal foods which had residues were muscular fibers and liver, but their residues occurring in feces have no structures which could possibly be confused with parasites.

5. Remnants from plant foods included pieces of plant tissue, which should not lead to confusion, and single undigested cells, which might cause confusion. Of these, single parenchyma cells of leafy vegetables, roots, and legumes, epidermal gland cells, epidermal hairs, bast fibers, and aleurone granules are the most common elements likely to be mistaken for parasites.

6. Though many foods have confusing residues in the feces, there are sufficient differences in structure, including their inconstancy of size and shape, their irregular walls, their lack of nuclei and internal organization, to provide differential diagnosis.

I wish to acknowledge with gratitude the kindly council and guidance of Professor Ernest Carroll Faust.

REFERENCES

Brug, S. L.: On Particular Binucleate Bodies Often Found in Human Feces, Ann. Trop. Med. **30:** 33, 1936.

Cammidge, P. J.: The Feces of Children and Adults, New York, 1914, William Wood & Co., page 25.

Faust, E. C.: Anomalies Found in Fecal Examinations in China, Chinese M. J. **38:** 820, 1924.

Hensel, O., Weil, R., and Joliffe, S. W.: The Urine and Feces in Diagnosis, Philadelphia, 1905, Lea & Febiger, page 1.

Hülsebosch, M. L. Q. van Ledden: Macro-und Microscopische Diagnostik der Menschlichen Exkremente, Berlin, 1899, J. Springer, page 1.

Patterson, S. W.: The Microscopic Examination of Feces, Lancet **2:** 785, 1929.

Schmidt, A., and Strassburger, J.: Die Faeces des Menschen im Normalen und Krankhaften Zustunde, Berlin, 1905, A. Hirschwald, page 45.

Stevens, W. C.: Plant Anatomy, Philadelphia, 1907, P. Blakiston's Son & Co., page 1.

Thompson, J. G., and Robertson, A.: Fish as the Source of Certain Coccidia Recently Described as Intestinal Parasites of Man, Brit. M. J. **1:** 282, 1926.

Winton, A. L.: The Microscopy of Vegetable Foods, New York, 1906, John Wiley & Sons, page 1.

THE PATHOLOGIC TISSUE CHANGES PRODUCED BY SULFATHIAZOLE AND SULFATHIAZOLINE IN RABBITS*

JOHN A. KOLMER, M.D., PHILADELPHIA, PA.

WITH THE TECHNICAL ASSISTANCE OF ANNA M. RULE AND LORAINE GROSKIN

AS SHOWN by Rake, Van Dyke and Corwin,[1] the toxicity of sulfathiazole and sulfapyridine, including the pathologic tissue changes produced by oral administration, varies in mice, rats, and monkeys. Thus sulfathiazole was found more toxic for mice than sulfapyridine with the production of more pronounced tissue changes in the spleen and kidneys. In rats, however, sulfapyridine was found more toxic than sulfathiazole, with the production of more marked lesions in the kidneys. The most pronounced pathologic tissue changes produced by both compounds were observed in the kidneys due to the deposition of crystals in the collecting tubules with the production of uroliths resulting in obstruction associated in some instances with necrosis of the tubular epithelium. The spleen probably ranked next in histologic injury, largely confined to the germinal centers of the Malpighian corpuscles with hyperplastic changes sometimes associated with pyknosis of the surrounding lymphoblastic cells and their ultimate disappearance. The liver was less frequently involved, with the production of cloudy swelling sometimes associated with fatty infiltration and hyaline necrosis, while the lungs, brain, and meninges have uniformly shown no gross or microscopic evidences of injury.

In view of the importance of including gross and microscopic examinations of the organs in connection with studies bearing upon the toxicity of chemotherapeutic compounds, we have thought it advisable to determine the chemopathologic effects of sulfathiazole in rabbits by oral administration of the compounds; also of 2-sulfanilyl-3-5 dihydrothiazole (sulfathiazoline), a new thiazole compound synthesized by Raiziss and Clemence,[2] and reported by Raiziss, Severac, and Moetsch[3] as being of low toxicity for mice, rabbits, and dogs with therapeutic effects about the same as sulfathiazole in the treatment of experimental types II and III pneumococcal infections of mice and superior to sulfathiazole in the treatment of experimental staphylococcal infections of mice.

EXPERIMENTAL

Normal adult rabbits were employed. In order to insure accurate dosage each compound was administered orally in capsules twice daily (10:00 A.M. and 3:00 P.M.) in doses of 0.050, 0.100, and 0.200 Gm. per kilogram of weight,

*From the Research Institute of Cutaneous Medicine, Philadelphia.
Received for publication, July 3, 1941.

for ten days in succession (20 doses), four animals being used for each dose. These amounts totaled 0.1, 0.2, and 0.4 Gm. per kilogram daily, equivalent to 7 to 28 Gm. per 70 kg. of weight daily over a period of ten days.

TABLE I

PATHOLOGIC TISSUE CHANGES PRODUCED IN RABBITS BY THE ORAL ADMINISTRATION OF SULFATHIAZOLE

NO.	WEIGHT (GM.)	DOSE PER KILO* (GM.)	RESULTS	PATHOLOGIC TISSUE CHANGES			
				KIDNEYS	LIVER	SPLEEN	BRAIN, CORD, AND MENINGES
1	1,950	0.050	Survival	–†	–†	–†	–†
2	1,800	0.050	Survival	–	–	–	–
3	1,800	0.050	Survival	–	–	–	–
4	2,000	0.050	Survival	–	–	–	–
5	2,000	0.100	Survival	+‡	–	+¶	–
6	2,100	0.100	Survival	–	–	–	–
7	1,900	0.100	Survival	+	+§	+	–
8	2,150	0.100	Survival	–	–	+	–
9	2,100	0.200	Survival	+	–	+	–
10	1,700	0.200	Survival	++	++	++	–
11	1,900	0.200	Survival	+	+	+	–
12	2,150	0.200	Survival	+	–	+	–

*Orally in capsules twice daily for ten days (20 doses).

†– = No gross or microscopic changes from the normal.

‡+ = Dilatation of the convoluted tubules with crystals; ++ = dilatation of the convoluted and collecting tubules with some necrosis of the tubular epithelium and the formation of cellular casts; congestion with blood in Bowman's capsules.

§+ = Slight cloudy swelling; ++ = cloudy swelling and some fatty infiltration associated with slight peripheral necrosis.

¶+ = Slight hyperplastic changes in the germinal centers of the Malpighian corpuscles; ++ = same with pyknosis of the surrounding lymphoblastic cells.

TABLE II

PATHOLOGIC TISSUE CHANGES PRODUCED IN RABBITS BY THE ORAL ADMINISTRATION OF SULFATHIAZOLINE

NO.	WEIGHT (GM.)	DOSE PER KILO* (GM.)	RESULTS	PATHOLOGIC TISSUE CHANGES			
				KIDNEYS	LIVER	SPLEEN	BRAIN, CORD, AND MENINGES
1	1,800	0.050	Survival	–†	–†	–†	–†
2	1,900	0.050	Survival	–	–	–	–
3	2,000	0.050	Survival	–	–	–	–
4	1,800	0.050	Survival	–	–	–	–
5	1,700	0.100	Survival	–	–	–	–
6	1,800	0.100	Survival	+‡	+§	–	–
7	1,600	0.100	Survival	+	–	+¶	–
8	1,950	0.100	Survival	+	–	+	–
9	2,100	0.200	Survival	++	++	+	–
10	1,700	0.200	Survival	+	–	–	–
11	1,700	0.200	Survival	++	+	++	–
12	1,700	*	*	+	–	–	–

*Orally in capsules twice daily for ten days (20 doses).

†– = No gross or microscopic changes from the normal.

‡+ = Dilatation of the convoluted tubules with crystals; ++ = dilatation of the convoluted and collecting tubules with some necrosis of the tubular epithelium and the formation of cellular casts; congestion with blood in Bowman's capsules.

§+ = Slight cloudy swelling; ++ = cloudy swelling and some fatty infiltration associated with slight peripheral necrosis.

¶+ = Slight hyperplastic changes in the germinal centers of the Malpighian corpuscles; ++ = same with pyknosis of the surrounding lymphoblastic cells.

As shown in Tables I and II, all animals survived in the case of both compounds. There was no diarrhea or other clinical evidence of toxicity, except

some loss of weight in the case of the 8 animals receiving the largest doses of both compounds.

One week after the last dose all animals were sacrificed with macroscopic examination of the kidneys, liver, spleen, brain, spinal cord, and meninges. Sections were also prepared of these organs and subjected to microscopic examination.

RESULTS

The results are summarized in Tables I and II. It will be observed that neither compound in dose of 0.05 Gm. per kilogram twice daily showed any gross or microscopic evidences of injury of the organs studied. Both, however, have shown evidences of injury of the kidneys, liver, and spleen in dose of 0.1 Gm. per kilogram twice daily in the case of some animals which were more frequent, and sometimes more pronounced in those given 0.2 Gm. per kilogram twice daily. No gross or microscopic evidences of injury were found in the meninges, brain, and spinal cords of any of the animals, including those receiving the largest amounts of both compounds.

The usual changes in the kidneys consisted of slight enlargement with hyperemia due to dilatation of the convoluted or collecting tubules sometimes associated with tubular necrosis, cellular casts, and occasionally blood in the capsules of Bowman. The latter changes were found only in rabbits receiving the largest doses of both compounds.

The spleen was next in the frequency of tissue injury, and especially in the case of those animals receiving the largest amounts of both compounds. When involved, it was slightly enlarged and hyperemic, usually showing upon microscopic examination some hyperplasia of the germinal centers of the Malpighian corpuscles occasionally associated with congestion and with pyknosis of the surrounding lymphoblastic cells.

Both compounds in dose of 0.1 Gm. per kilogram twice daily showed only occasionally a slight cloudy swelling of the lobules of the liver. In the case of animals given 0.2 Gm. per kilogram twice daily, however, the degree of cloudy swelling was more pronounced, sometimes associated with fatty infiltration and slight necrosis of the peripheral cells of the lobules. There were no evidences, however, of injury or obstruction of the biliary ducts.

Like sulfapyridine, it would appear, therefore, that the main injury from sulfathiazole and sulfathiazoline is to be found in the kidneys, mainly due to obstruction of the tubules by crystals of the acetylated compounds. This may result in hydronephrosis, with enlargement of the organs which may progress in some instances to tubular necrosis.

Insofar as comparative results of the two compounds are concerned, it would appear that both are of extremely low toxicity for rabbits by oral administration and of about equal toxicity insofar as injury of the kidneys, liver, and spleen are concerned. As previously stated, neither compound showed any clinical, macroscopic, or microscopic evidences of injury of the central nervous system.

SUMMARY

1. Both sulfathiazole and sulfathiazoline are of low toxicity for rabbits by oral administration. Doses of 0.05, 0.1, and 0.2 Gm. per kilogram twice daily for ten days in succession (20 doses) were well borne with no fatalities.

2. Neither compound showed any macroscopic or microscopic evidences of injury of the meninges, brain, or spinal cord.

3. In dose of 0.05 Gm. per kilogram twice daily for 20 doses neither compound showed any gross or microscopic injuries of the kidneys, liver, or spleen.

4. Both compounds in dose of 0.1 and 0.2 Gm. per kilogram twice daily for 20 doses showed injury of the kidneys largely due to obstruction of the tubules by crystals of acetylated compounds, sometimes associated with tubular necrosis. These were the most frequent lesions observed. Both compunds were about the same in their production.

5. Both compounds in dose of 0.1 and 0.2 Gm. per kilogram twice daily for 20 doses were about equal in the production of injury of the liver, usually expressed as cloudy swelling sometimes associated with slight fatty degeneration and necrosis of the peripheral cells of the lobules.

6. Both compounds in dose of 0.1 and 0.2 Gm. per kilogram twice daily for 20 doses were about equal in the production of injury of the spleen, usually expressed by slightly hyperplastic changes in the germinal centers of the Malpighian corpuscles.

REFERENCES

1. Rake, G., Van Dyke, H. B., and Corwin, W. C.: Pathologic Changes Following Prolonged Administration of Sulfathiazole and Sulfapyridine, Am. J. M. Sc. 200: 353, 1940.
2. Raiziss, G. W., and Clemence, L. W.: 2-Sulfanilyl-Aminothiazoline. Personal communication. To be published.
3. Raiziss, G. W., Severac, M., and Moetsch, J. C.: Chemotherapeutic Studies of 2-Sulfanilyl-3-5 Dihydrothiazole (Sulfathiazoline). Personal communication. To be published.

SULFANILAMIDE FOR THE TREATMENT OF GONORRHEA OF THE ANAL CANAL

ROBERT TURELL, M.D., NEW YORK, N. Y.

WITH THE TECHNICAL ASSISTANCE OF MIRIAM GREEN

IN A previous report[1] I emphasized that sulfanilamide administered orally is effective in the treatment of anal gonorrhea in the absence of localized suppurations or poorly draining sinuses. The plan of treatment consisted of the administration of 1 Gm. of sulfanilamide every four hours for 24 to 30 doses, which resulted in a concentration of from 6 to 11 mg. of free sulfanilamide in each 100 c.c. of blood. Thereafter, the drug was reduced to from 3 to 4 Gm. daily and was continued for a period of from four to five weeks. Berck,[2] using smaller amounts of sulfanilamide, has also noted good results in the treatment of this lesion.

The following case histories are illustrative of the effectiveness of sulfanilamide* therapy for gonorrhea of the anal canal.

Received for publication, July 8, 1941.

*Sulfathiazole or sulfadiazine is now preferred.

CASE 1.—A 34-year-old white woman was seen on March 22, 1938, with intermittent discomfort of the anus and occasional sharp pain on defecation. About three years before, she had received intensive treatment for genital gonorrhea. Proctologic examination now revealed a superficial fissure with a deep, reddened, infected, and edematous crypt of Morgagni on the posterior arc of the anal canal. Material obtained from the interior of the involved crypt with the aid of a bent platinum wire loop (which was inserted through small anal speculum) revealed numerous leucocytes and gram-negative intracellular diplococci resembling gonococci. The sedimentation time was above normal. Six grams of sulfanilamide were given daily by mouth for five days; 4 Gm. daily for the succeeding eleven days, and thereafter 3.3 Gm. daily for a period of over five weeks. Instrumental examination was avoided for a period of seventeen days. At the end of that time, as well as during subsequent re-examinations, no gram-negative intracellular diplococci were observed. The patient remained cured when last seen on May 12, 1939.

CASE 2.—A 30-year-old white woman had received an undetermined amount of sulfanilamide by mouth (the exact amount of the drug taken could not be ascertained) and azosulfamide solution parenterally in June, 1937, for the treatment of genital gonorrhea. On Dec. 16, 1938, she had complained of sharp shooting pains in the muscles of the thighs and in various joints. The physical examination was normal, except for reddening, injection, and tenderness of the anal canal and lower rectum. Thin mucopurulent material obtained from an anteriorly situated inflamed crypt of Morgagni showed gram-negative intracellular diplococci. On Sept. 29, 1939, tenderness in the region of the right Bartholin gland was observed, which was relieved by external heat. On Jan. 15, 1940, the patient again complained of pain in the muscles of the left thigh; this persisted intermittently in spite of conservative therapy (hot hip baths, salicylates). Reexamination on May 1, 1940, showed injection of the anal canal. A Gram stain of the material obtained from the interior of an anteriorly situated crypt of Morgagni again revealed gram-negative intracellular diplococci and many leucocytes. Sulfanilamide therapy was begun on May 25, giving 4.5 Gm. daily for the first four days, and 2 to 4 Gm. daily thereafter until a total of 50 Gm. of sulfanilamide had been taken. Re-examination of the anal canal on June 14, and again on July 22, showed a few leucocytes per field; no gram-negative intracellular diplococci were found. The patient has remained symptom-free to date.

COMMENT

These results show that sulfanilamide appears to be effective in the treatment of uncomplicated gonorrhea of the anal canal. The plan of sulfanilamide therapy employed has been found safe for the patient who is under daily medical supervision.

Gonorrheal proctitis is "a disease one sees only if one looks for it" (Jullien-Martin). It is realized that gonococci may remain alive and virulent for years in the anal structures,[3] and that reinfection of the genital tract may occur from the anal focus.[4-7] Except for cases of pederasty, contamination in girls and women is believed to take place during the act of defecation, when a simultaneous expulsion of gonococci-laden discharge from the vagina and a normal eversion of the anal mucous membrane occur. Suitable biologic conditions for the inception and maintenance of gonorrheal infection are furnished by the transitional epithelium of the intermediate zone of the anal canal,[6] the anal ducts, and the crypts of Morgagni.[8] The infection may spread to the deeper perianal and perirectal structures through the anal ducts which lead to racemose multiglandular structures located in the perianal tissues, and penetrate the sphincter muscles, especially the internal sphincter,[9-11] producing suppurations and subsequent fistulas.

SUMMARY

Sulfanilamide has been successfully employed in the treatment of uncomplicated gonorrhea of the anal canal.

REFERENCES

1. Turell, R.: Sulfanilamide for Treatment of Gonorrhea of the Anal Canal. Read before the New York Proctologic Society, New York, N. Y., Nov. 9, 1939.
2. Berek, M.: Personal communication to the author.
3. Bichel, L., and Abraham, L., cited by Rosser, C.: Current Questions in Proctology, J. A. M. A. **101**: 1043, 1933.
4. Martin, C. L.: Rectal Gonorrhea in Women, J. A. M. A. **104**: 192, 1935.
5. Brunet, W. M., and Salberg, J. B.: Gonococcus Infection of the Anus and Rectum in Women: Its Importance, Frequency, and Treatment; Study of 250 Cases, Am. J. Syph., Gonor. & Ven. Dis. **20**: 37, 1936.
6. Tucker, C. C., and Hellwig, C. A.: Anal Ducts, Arch. Surg. **31**: 521, 1935.
7. Black, C. V.: Gonorrheal Infections in Female, J. Kansas M. Soc. **36**: 56, 1935.
8. Rosser, C.: Clinical Variations in Negro Proctology; Venereal Factor, J. A. M. A. **87**: 2084, 1926.
9. Herrmann, G., and Desfosses, L.: Sur la muqueuse de la région cloacale du rectum, Compt. rend. Acad. d. sc. **90**: 1301, 1880.
10. Johnson, F. P.: The Development of the Rectum in the Human Embryo, Am. J. Anat. **16**: 1, 1914.
11. Harris, H. A.: Discussion on Fistula-in-Ano; Some Embryological Aspects of Problem Involved, Proc. Roy. Soc. Med. (Sect. Surg., Sub-Section Proct.) **22**: 43, 1929.

876 Park Avenue

BACTERIUM COLI ANAEROGENES SEPTICEMIA*

Report of a Case With Recovery

H. Jacob Flax, M.D., and Frederick W. Shaw, M.D.
Richmond, Va.

THE incidence of infection with *Bacterium coli anaerogenes* is very small, and only a few cases of septicemia produced by this organism have been reported. Lembke[1] in 1896 originally described this organism which he recovered from dog dejecta and named it *B. coli anaerogenes*. In 1900 Migula,[2] in his *System of Bacteriology*, called it *Bacterium lembkei*. Apparently, the same organism was reported by Castellani[3] in 1907 as *Bacillus ceylonensis;* he recovered it in four cases of a febrile disease in Ceylon. In two of these cases he obtained the organism by blood culture. Wilson[4] the next year isolated such an organism in six cases of cystitis. Houston[5] in 1906, and Swan[6] in 1909, found similar bacteria in the stools of healthy persons. In 1906 Dudgeon[7] isolated a pathogenic bacillus of this type from the secretion of an enlarged prostate gland. Also in this year similar organisms were found by Catheart[8] among the flora of "blown" tins of preserved food. Nabarro[9] in 1923 isolated from cases of epidemic and other forms of diarrhea, which occurred mainly in children, a group of bacteria which he called *B. coli anaerogenes*. However, some of these strains were later shown by Kerrin[10] in 1928

*From the Stuart Circle Hospital and the Department of Bacteriology and Parasitology, Medical College of Virginia, Richmond.
Received for publication, July 16, 1941.

to be identical culturally and serologically with the *B. dysenteriae Sonne*. Bamforth[11] in 1923 concluded in his studies that it was serologically related to some other members of the coli-dysentery group, but not to the Sonne organism. In 1939 Savino and co-workers[12] demonstrated the presence of bacteria of the *Coli anaerogenes* group in gray rats caught in Buenos Aires.

The organism under discussion is known by several other names:[13-14] *Bacterium anaerogenes chester*, *Bacillus belfastiensis*, *Castellanus coli anaerogenes*, *Escherichia anaerogenes*, and *Eberthella belfastiensis*. *Bacterium coli anaerogenes*, according to Bergey,[13] is a small, nonmotile, gram-negative rod. On agar the growth is slow, producing circular, grayish-white, smooth colonies on plates, and a grayish-white streak on the slant. There is no liquefaction in the gelatin stab; litmus milk is turned acid and coagulates; broth is made turbid; a brown growth is produced on potato, and indol is formed. Acid, but no gas, is observed in dextrose, levulose, arabinose, lactose, maltose, sucrose, raffinose, mannitol, sorbitol, and isosorbitol; no acid from inulin, salicin, dulcitol, glycerol, or adonitol. It is a facultative anaerobic, growing best at 37° C., and is found in the intestinal tract of man where it may produce symptoms that resemble those of typhoid fever. Ford[14] stated that *B. coli anaerogenes* is virulent to guinea pigs: one cubic centimeter of a twenty-four-hour broth culture will produce enteritis, peritonitis, congestion of the liver and lungs, and cloudy swelling of the kidneys.

REPORT OF CASE

A white woman, aged 47 years, was admitted to Stuart Circle Hospital with menstrual bleeding of three months' duration. Pelvic examination revealed a large tumor, which filled the right side of the pelvis. A diagnosis of multiple fibroids of the uterus was substantiated at operation two days following admission, but there was also found a large intraligamentous ovarian cyst of the multilocular type which filled the pelvis. The left ovary appeared normal. Both tubes were in a state of hydrosalpinx. The surgery undertaken consisted of a right oophorectomy, supravaginal hysterectomy, bilateral salpingectomy and a prophylactic appendectomy; the abdomen was closed without drainage. The patient left the operating room in good condition.

The postoperative course was marked by considerable nausea and vomiting for which the patient received parenterally solutions of dextrose and normal saline. Abdominal distention was relieved by a continuous suction apparatus attached to an inlying duodenal tube and by the low gravity method of colonic irrigation. During the first postoperative day (beginning twenty-four hours after operation) the temperature rose to 100° F. and on the second day it became suddenly elevated to 105.4° F. A severe chill was experienced, and the pulse rate reached 120 per minute. That night, and the following day, the temperature gradually came down to 100° F. On this, the third postoperative day, a transfusion of 500 c.c. of blood was given by the direct method. Blood was taken for a culture, and this was repeated the following morning. From both of these there was recovered a small, nonmotile, gram-negative rod, which could not be readily identified. During the next two days the patient had repeated chills at which times the temperature went as high as 104° F.

Sulfanilamide, 15 grains every four hours, was started on the fifth postoperative day. The night of the sixth day the patient was suddenly stricken with an attack of dyspnea, chills, and a temperature of 105° F. She appeared cyanotic; the heart action was accelerated, and the blood pressure increased from systolic 150 to 200 mm. Hg in less than an hour. The heart and lungs were found to be clear to auscultation and percussion. Since a pulmonary embolus was suspected, the patient was placed in an oxygen tent. Her dyspnea was benefited and her color returned to normal under oxygen therapy and was maintained for two

days. However, the chills and fever (maximum 105° F.) continued despite all therapy. A pelvis pyelophlebitis was next considered as a possible cause of these symptoms. Sulfathiazole was then given orally instead of sulfanilamide, but this was also discontinued in thirty-six hours, after 120 grains were ingested without any demonstrable relief. Although some pneumonitis was seriously suspected, no definite lung findings could be detected, except for a few hypostatic râles in both bases. A roentgenogram of the chest was not taken. On the eighth day a second transfusion of 500 c.c. of whole blood was given without any apparent improvement in the patient's condition. The next day a widespread herpes labialis became evident, and this caused the patient much discomfort. The chills and fever (maximum 103.5° F.) continued with varying intensity until the thirteenth postoperative day. On the fourteenth day the stitches were removed, revealing a well-healed wound free of infection. The patient improved gradually under symptomatic treatment and was discharged as cured on the thirty-second day following her operation.

Throughout the period of infection the leucocyte count remained relatively low, between 8,000 and 10,000 cells per cubic millimeter, and reached 14,700 cells per cubic millimeter only once—on the tenth day. The hemoglobin on admission was 67 per cent (Newcomer), and during the postoperative period it dropped to 45 per cent; it finally reached 58 per cent before the patient left the hospital. The sedimentation index on the ninth postoperative day was 24 mm. in sixty minutes. The urine contained albumin in varying amounts, from a faint trace to four plus on the eighth postoperative day; it finally became negative on the fifteenth day. Five days following the patient's discharge, a report was received from the Virginia State Board of Health, which stated that the organism isolated from the blood culture was classified as belonging to the para-colon group, culturally resembling *Bacterium coli anaerogenes* in that it failed to produce gas in certain carbohydrates. A similar report was received through the Virginia State Board of Health from the New York State Board of Health and was verified by one of us (F. W. S.). Two months following her discharge, the patient returned to the hospital for a physical examination, at which time blood for agglutinations was taken. A positive titer of 1:1,500 dilution against *Bacterium coli anaerogenes* was discovered.

COMMENT

The focus of infection in this case was not determined. Since the *Bacterium coli anaerogenes* is an inhabitant of the intestinal tract in some people, it would seem logical to assume that the primary focus of infection was in the lymphatic vessels leading from the intestinal canal. From here, because of lowered resistance following the operation and the initial anemia which was still further lowered owing to the loss of blood during the operation, the bacteria might readily have spread through the lymphatic channels into the blood stream where a secondary focus could have been set up in the congested pelvic veins resulting in pyelophlebitis, and finally a septicemia. The patient was so overwhelmingly toxic throughout her febrile course that she was not expected to live on several occasions. Neither the blood transfusions nor the sulfonamide preparations (sulfanilamide, 90 grains, and sulfathiazole, 120 grains) seemed to have any therapeutic effect in shortening the course of the infection.

REFERENCES

1. Lembke, W.: Beitrag zur Bakterienflora des Darms, Arch. f. Hyg. **26**: 293, 1896.
2. Migula, W.: System der Bakterien, Jena, 1900, Gustav Fischer, Vol. 2, page 417.
3. Castellani, A.: Cases of Fever Confounded With Typhoid (Colitis With Pyrexia-Bacillus Ceylonensis), J. Hyg. **7**: 1, 1907.
4. Wilson, W. J.: Bacteriological Observations on Colon Bacilli Infecting the Urinary Tract With Special Remarks on Certain Colon Bacilli of the "Anaerogenes" Class, J. Hyg. **8**: 543, 1908.
5. Houston, A. C.: Report on the Bacteriological Examination of the Normal Stools of Healthy Persons, Report Medical Office, Local Government Board, 1902-1903, London **8**: 875, 1904.

6. Swan, J. M.: Bacterium Anaerogenes Isolated From Human Feces, J. Trop. Med. **12**: 239, 1909.
7. Dudgeon, L. S.: A New Pathogenic Bacillus Isolated From an Enlarged Prostate Gland, J. Hyg. **6**: 296, 1906.
8. Cathcart, E. P.: The Bacterial Flora of Blown Tins of Preserved Food, J. Hyg. **6**: 248, 1906.
9. Nabarro, D.: On Bacillus Coli Anaerogenes, J. Path. & Bact. **26**: 429, 1923.
10. Kerrin, J. C.: An Investigation of Bacillus Dysenteriae (Sonne Type III), J. Hyg. **28**: 4, 1928.
11. Bamforth, J.: An Enquiry Into Coli Anaerogenes Bacteria, J. Hyg. **34**: 69, 1934.
12. Savino, E., Villazon, N. M., and Anchezar, B.: Presence of Bacteria of Coli Anaerogenes Group in Gray Rats, Rev. d. Inst. bact. **9**: 142, 1939.
13. Bergey, D. H., and Others: Manual of Determinative Bacteriology, ed. 5, Baltimore, 1939, Williams & Wilkins Company.
14. Ford, W. W.: Textbook of Bacteriology, Philadelphia, 1927, W. B. Saunders Co.

THE ANTAGONISM BETWEEN ANESTHETIC STEROID HORMONES AND PENTAMETHYLENETETRAZOL (METRAZOL)*

HANS SELYE, M.D., PH.D., F.R.S.C., MONTREAL, CANADA

IT IS well known that analeptic, convulsive drugs, such as picrotoxin or pentamethylenetetrazol (metrazol), counteract the narcotic effect of certain anesthetics, especially the barbiturates and tribromethanol (avertin). Since the relevant literature has recently been reviewed in detail by Beecher,[1] it will not be necessary to discuss it here. It has also been found that some steroid compounds, especially those endowed with hormone action, act as anesthetics if given intraperitoneally or intravenously, that is to say, if administered in a manner which permits the rapid establishment of a high steroid concentration in the blood.[2-7] This type of anesthesia produced by naturally occurring hormonal substances proved very satisfactory for use in laboratory animals, since the safety margin between the anesthetic and lethal dose is comparatively great, and the anesthesia is accompanied by very pronounced muscular relaxation. Steroid anesthesia is especially suitable when long-lasting narcosis is required, but it cannot be used satisfactorily in cases in which a deep anesthesia of short duration is needed because a dose sufficient to produce deep anesthesia, at any one time in the course of its action, usually maintains the state of narcosis for several hours. It appeared of interest, therefore, to establish whether pentamethylenetetrazol could arouse animals from the hormone anesthesia whenever desired.

EXPERIMENTAL

In our first experiment young male albino rats were divided into three equal groups. In the first two groups deep anesthesia was produced by the intraperitoneal administration of desoxycorticosterone acetate (D.C.A.) in peanut oil solution. Thirty minutes after the injection of this hormone, when all animals were in complete narcosis, one of the groups pretreated with desoxycortico-

*From the Department of Anatomy, McGill University, Montreal.
This investigation was supported by a grant from the Committee on Research in Endocrinology, National Research Council.
Received for publication, July 18, 1941.

sterone acetate and a group of controls not pretreated received a subcutaneous injection of pentamethylenetetrazol in aqueous solution. Table I summarizes the results of this experiment.

TABLE I

ANTAGONISM BETWEEN DESOXYCORTICOSTERONE ACETATE AND PENTAMETHYLENETETRAZOL

TREATMENT	NO. OF ANIMALS	AVERAGE BODY WEIGHT IN GM.	AVERAGE DEGREE OF ANESTHESIA	CONVULSIONS	DEATHS
Desoxycorticosterone acetate 4 mg. per 0.2 ml.	7	35	+ + +	0	0
Pentamethylenetetrazol 5 mg. per 0.2 ml. and Desoxycorticosterone acetate 4 mg. per 0.2 ml.	7	36	Trace	0	1
Pentamethylenetetrazol 5 mg. per 0.2 ml.	7	36	0	+ + +	7

Our data clearly indicate that pentamethylenetetrazol antagonizes the anesthetic action of desoxycorticosterone acetate. It should be stated that this is not merely a prevention but an actual interruption of the anesthesia, since the animals in the group in which desoxycorticosterone acetate was administered before the pentamethylenetetrazol, were deeply anesthetized at the time the latter drug was administered, yet five minutes later they were on their feet again and showed only slight traces of a narcosis, such as uncertainty of their movements and unwillingness to run about. It is even more interesting to note that in the condition of hormone anesthesia, otherwise lethal doses of pentamethylenetetrazol did not kill a single animal and indeed prevented even the appearance of convulsions. The animals not pretreated, which died from pentamethylenetetrazol overdosage, developed very pronounced lung edema. This was also prevented by desoxycorticosterone acetate. Thus we can see that the antagonism between desoxytorticosterone acetate and pentamethylenetetrazol is mutual.

TABLE II

ANTAGONISM BETWEEN PROGESTERONE AND PENTAMETHYLENETETRAZOL

TREATMENT	NO. OF ANIMALS	AVERAGE BODY WEIGHT IN GM.	AVERAGE DEGREE OF ANESTHESIA	CONVULSIONS	DEATHS
Progesterone 5 mg. per 0.2 ml.	10	45	+ + +	0	0
Pentamethylenetetrazol 3 mg. per 0.2 ml. twice and progesterone 5 mg. per 0.2 ml.	12	47	0	0	0
Pentamethylenetetrazol 3 mg. per 0.2 ml. twice	10	46	0	+ + +	10

A second series of experiments was performed under very similar conditions in order to establish whether progesterone, which is also a potent anesthetic when given by the intraperitoneal route, would likewise prove to be an antagonist of pentamethylenetetrazol. The results of this experiment are summarized in Table II.

In this experiment pentamethylenetetrazol was administered in two injections. The first dose of 3 mg. aroused the animals from progesterone anesthesia

for a period of about fifteen minutes, after which they began to relapse into narcosis. Permanent awakening was produced, however, by a second dose of 3 mg. The controls not pretreated showed marked convulsions even under the influence of the first injection of pentamethylenetetrazol, and one animal died from lung edema. The second injection produced fatal lung edema in all the remaining controls.

DISCUSSION

Our experiments indicate that pentamethylenetetrazol can interrupt steroid anesthesia so that in this respect the narcosis produced by progesterone and desoxycorticosterone acetate resembles that elicited by barbiturates or tribromethanol. It is also evident that the convulsions and the fatal lung edema usually produced by lethal doses of pentamethylenetetrazol are very effectively prevented by steroid anesthetics. In this respect the steroids again resemble the barbiturates which, according to Maloney[8] likewise raise resistance to convulsive drugs. The anesthetic steroids and pentamethylenetetrazol may be regarded as potent mutual antagonists of each other's pharmacologic actions.

SUMMARY

Experiments on young albino rats indicate that the anesthesia produced by the intraperitoneal administration of steroid hormones (progesterone or desoxycorticosterone acetate) can be interrupted at will by pentamethylenetetrazol (metrazol). Conversely, fatal doses of pentamethylenetetrazol are readily tolerated without causing either convulsions or lung edema by rats receiving suitable doses of anesthetic steroid hormones.

I am indebted to Doctors Gregory Stragnell and Erwin Schwenk, of the Schering Corporation, New Jersey, for the progesterone and desoxycorticosterone acetate, and to Dr. Fred B. Western, of the Bilhuber-Knoll Corporation, Orange, N. J., for the metrazol used in these experiments.

REFERENCES

1. Beecher, H. K.: Some Current Problems of Anesthesia, Surgery **7**: 931, 1940.
2. Selye, H.: The Anesthetic Effect of Steroid Hormones, Proc. Soc. Exper. Biol. & Med. **46**: 116, 1941.
3. Selye, H.: On the Role of the Liver in the Detoxification of Steroid Hormones and Artificial Estrogens, J. Pharmacol. & Exper. Therap. **71**: 236, 1941.
4. Selye, H.: Acquired Adaptation to the Anesthetic Effect of Steroid Hormones, J. Immunol. **41**: 259, 1941.
5. Selye, H.: Studies Concerning the Anesthetic Action of Steroid Hormones, J. Pharmacol. & Exper. Therap. **73**: 127, 1941.
6. Winter, H., and Selye, H.: Conditions Influencing the Course of Steroid Hormone Anesthesia, Am. Physiol. Soc. Meeting, Chicago, April, 1941.
7. Winter, H.: Conditions Influencing the Course of Steroid Hormone Anesthesia, Endocrinology **29**: 790, 1941.
8. Maloney, A. H.: Analysis of Stimulant-Depressant Action of Caffeine, Coramine and Metrazol (Cardiazol) Based on Experimental Data (Study on Rats and Rabbits Narcotized With Medinal, Barbital Preparation), Arch. internat. de pharmacodyn. et de thérap. **52**: 373, 1936.

CLINICAL CHEMISTRY

PLASMA PROTEINS IN THERAPEUTIC FEVER*

ISRAEL KOPP, M.D., BOSTON, MASS.

CHANGES in the plasma proteins have been observed in various infections and have consisted in the main of a reduction in the albumin and increases in the globulin and fibrinogen.[1-5] A study of the factors responsible for the edema occasionally resulting during the course of therapeutic malaria has revealed also rather marked changes in the plasma protein fractions.[6]

The present communication is concerned with the changes in the plasma proteins of nine patients to whom malaria, artificial fever, and typhoid vaccine fever were administered for treatment of syphilis of the central nervous system.

Material.—Nine male patients, ranging from 40 to 51 years of age, were hospitalized for fever therapy of general paresis. Physical and laboratory procedures revealed no other complicating diseases.

Methods.—Six patients were given tertian malaria only; one patient, artificial fever and malaria; one patient, tertian and quartan malaria and typhoid vaccine fever; and one patient, typhoid vaccine fever.

Malarial therapy was administered by the intravenous injection of 3 to 4 c.c. of malarial blood. The incubation period ranged from one to seven days. True tertian or quartan fever was not encountered, the fever being of the mixed or quotidian type. Malaria was terminated in seven patients after 10 to 13 paroxysms by the daily administration of 30 grains of quinine sulfate for one week. One patient experienced only four paroxysms of fever above 104° R. after two inoculations with tertian malaria and one with quartan malaria.

Typhoid vaccine fever was induced by the intravenous injection of increasing doses of mixed typhoid vaccine (*Bacillus typhosus,* paratyphoid A and B), administered usually three times a week. One patient was given eleven bouts of fever, and a second, sixteen bouts of fever.

Artificial fever was induced by means of the inductotherm and humidified air cabinet. Body temperature was raised to level of 105° to 106° R., and prolonged for two hours at each treatment. A total of fourteen treatments was given to one patient over a thirty-eight-day period.

The daily hospital diet consisted roughly of the following: protein 80 Gm., carbohydrate 315 Gm., and fats 85 Gm. When malarial fever was present and food intake diminished, the daily diet was reinforced by the addition of at least one quart of milk and two eggs (protein 42 Gm., carbohydrate 50 Gm., and fat 52 Gm.). After termination of the malarial fever the patient was given a high vitamin and high caloric diet and 20 grains of ferrous sulfate daily.

*From the Boston Psychopathic Hospital, Dr. Harry C. Solomon, Chief of Therapeutic Research.

Received for publication, January 26, 1942.

Sodium chloride was administered to seven patients during fever therapy as a result of the observations of Judd,[7] who felt that patients were better able to tolerate malarial therapy when 10 grains of this salt were given on those days when body temperature rose above 103° R. Larger doses, ranging from 3 to 6 Gm. daily, were given to our patients. The sodium chloride was omitted when therapeutic fever was terminated with the exception of one patient, who continued to receive 6 Gm. daily for one week.

Studies were made of the plasma proteins, hematocrit, red blood cell, white blood cell, and hemoglobin (Sahli) levels on venous blood samples in the resting state. The total protein was obtained by the determination of the total nitrogen by a modified micro-Kjeldahl.[8] The nonprotein nitrogen was determined by the method of Folin and Wu,[9] and the difference between the total nitrogen and the nonprotein nitrogen was multiplied by the factor 6.25 to give the total protein. The albumin and globulin were determined by the sodium sulfate method of Howe.[10] The fibrinogen was determined by precipitation as fibrin by the method of Cullen and Van Slyke,[11] and the nitrogen was determined by the difference between the total nitrogen and the nitrogen in the fibrinogen-free filtrate. In the last series of studies the fibrinogen was precipitated as above, and the nitrogen content of the fibrin was determined directly. Potassium oxalate in the concentration of 2 mg. per cubic centimeter was used as the anticoagulant.

RESULTS

Malaria.—Intravenous malarial inoculation was followed in seven of eight patients by a slight or a marked and rapid fall in albumin during the incubation period and before any fever occurred. One patient experienced no fever for thirteen days following his first inoculation with tertian malaria. Albumin fell from 4.82 to 4.01 Gm. during this period. A second patient showed a reduction in albumin from 5.1 to 4.3 Gm. three days after inoculation with tertian malaria and one day before any fever resulted. Only one patient showed an increase in albumin, from 4.37 to 4.70 Gm. Globulin and fibrinogen showed fluctuating changes during the incubation period; the changes in globulin ranging from a reduction of 25 per cent to an increase of 45 per cent, and those of fibrinogen from a fall of 50 per cent to an increase of 300 per cent.

The onset and recurrence of the malarial fever were accompanied by a rapid, progressive, and marked reduction in albumin (Chart 1). The reductions in albumin ranged from 30 to 40 per cent, falling from prefebrile values of 3.67 to 5.1 Gm. (average 4.53 Gm.) to levels of 2.23 to 3.25 Gm. (average 2.89 Gm.). Globulin fluctuated considerably during malarial fever but showed no consistent trend. Increases of 11 to 65 per cent occurred in seven patients. In the eighth patient globulin values throughout malaria remained below the original level of 3.03 Gm., with a maximum reduction of about 32 per cent. Fibrinogen also showed no consistent trend during active malaria. Of the eight patients, one showed significant reductions as much as 50 per cent below prefebrile values; two, an increase and then a progressive fall below initial levels; two, fluctuations above and below original levels; and three, similar values or an increase.

The fall in albumin ceased immediately or within a few days after the administration of quinine and the cessation of malarial fever (Chart 1). Albumin values then rose rapidly, reaching prefebrile levels in six patients in fourteen to twenty-four days. In one patient the prefebrile value was reached only after seventy days. The malarial fever of the eighth patient ceased spontaneously, and no quinine was administered. Sixteen days after the last febrile reaction albumin differed only little from the lowest level obtained during

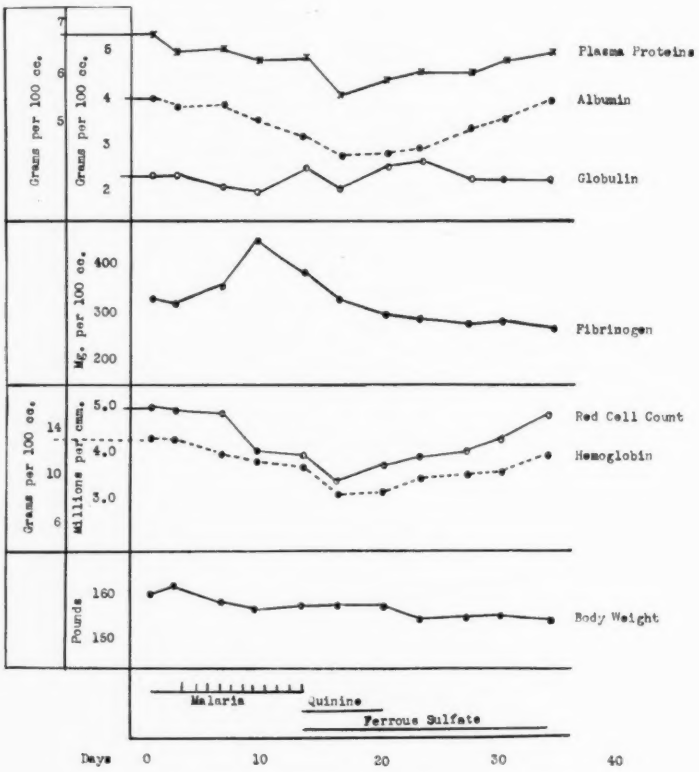

Chart 1.—Plasma proteins in therapeutic malaria.

malarial fever, namely, 3.6 and 3.23 Gm. Globulin remained increased for only a short period after malarial fever was terminated, and then fell slowly to reach prefebrile values in ten to eighteen days. The fibrinogen values returned to original levels fairly rapidly, in about seven days. Fibrinogen and globulin values of the patient who received no quinine therapy remained elevated even after two weeks' time.

Typhoid Vaccine Fever.—Only slight fluctuations in albumin, ranging from a fall of 9 per cent to an increase of 13 per cent, occurred in two patients to whom 11 and 16 bouts of typhoid vaccine fever were administered over periods of twenty-four and thirty-nine days, respectively (Chart 2). This is in marked contrast to the severe reductions of 30 to 40 per cent which were found during malaria. Globulin also showed fluctuations in both directions but of a smaller magnitude than in malaria. Both albumin and globulin values when treatment was terminated approximated those present before therapy. Fibrinogen in-

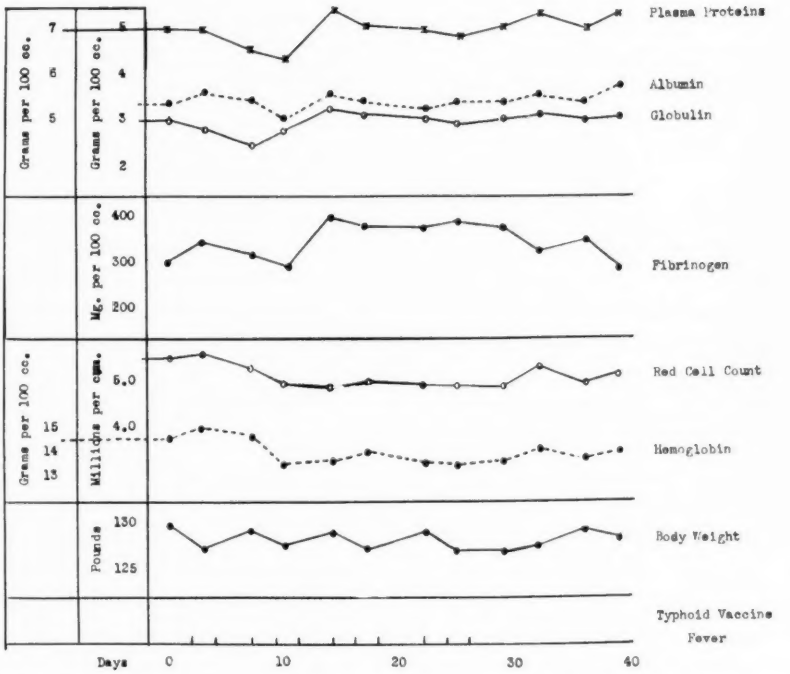

Chart 2.—Plasma proteins in fever induced by typhoid vaccine.

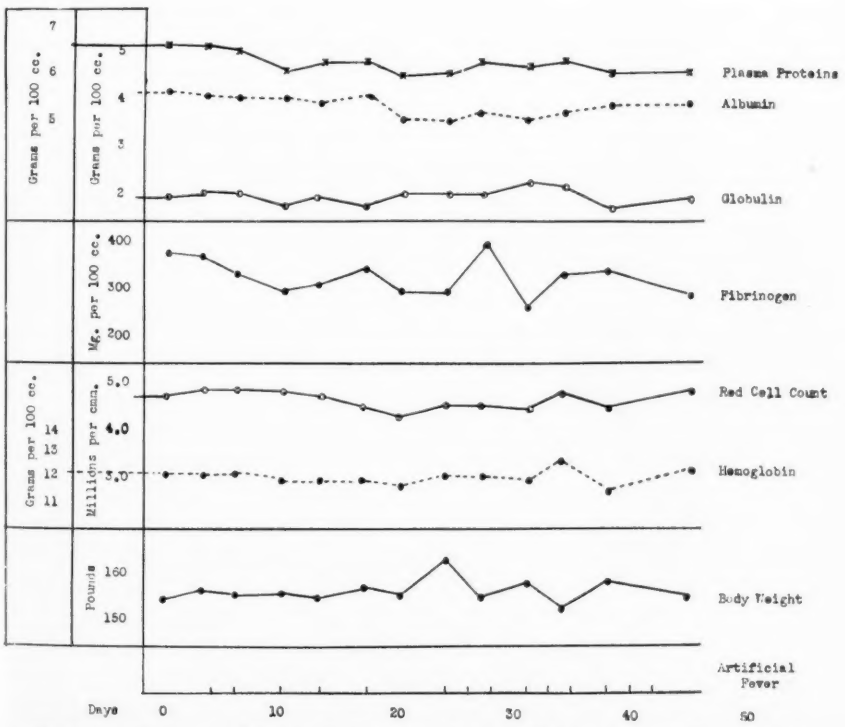

Chart 3.—Plasma proteins in fever induced by artificial means (inductotherm).

creased in both patients as a result of typhoid vaccine fever, in one 33 per cent and in the second 40 per cent. In the former fibrinogen returned to its original level within seven days after treatment ceased, but in the latter fibrinogen remained increased, more than 200 per cent, as late as thirty-one days thereafter.

Artificial Fever.—Repeated administration of artificial fever resulted in only a slight reduction of the albumin, the maximum fall being 13 per cent below the prefebrile level (Chart 3). The final value, however, after a course of 14 treatments, over a thirty-eight-day period, differed only little from that before treatment. The changes in globulin fluctuated equally, about 12 per cent, in both directions. Fibrinogen values during therapy tended to fall as much as 30 per cent below prefebrile levels but remained within normal limits. Three weeks after therapy was terminated the patient was inoculated with tertian malaria and experienced a 30 per cent reduction in albumin as a result of malarial fever (Chart 1).

DISCUSSION

The changes in the plasma protein fractions as a result of therapeutic malaria consist of a marked reduction in albumin, fluctuating changes in the globulin, and generally increased fibrinogen. Similar changes have been found in hookworm disease accompanied by severe anemia,[12] less marked changes in tuberculosis,[13] and other infections.[4] Lloyd and Paul,[1] by refractometric methods, found marked reductions in albumin and slight falls in the total globulin in patients with malignant and benign tertian malaria. The degree of albumin reduction seemed to parallel the intensity of the infection, greater reductions occurring in patients with high temperatures than in those with subfebrile infections. Similar changes were found by these authors in patients with typhoid fever. The rise in albumin to normal was said to be more rapid following malaria than typhoid fever, and was attributed to the presence of a specific drug, quinine, for the treatment of the former. It is possible that the absence of quinine therapy in one of our patients may be responsible for the only slight rise of albumin from its lowest level of 3.23 Gm. during malaria to 3.6 Gm. sixteen days after fever spontaneously ceased, and for its failure to rise to the original level of 4.82 Gm. during this period. Lloyd and Paul[1] observed several untreated patients with malaria in whom parasites were present in the peripheral blood stream but no fever was present. All showed the characteristic protein changes, a reduction in albumin, and variable changes in globulin.

The changes in the serum protein fractions as a result of malaria were much more pronounced than those found in fever produced either by mixed typhoid vaccine or by artificial means. This may be due to (a) the rapid recurrence and greater amount of fever; (b) the presence of blood destruction; and (c) the presence of an infectious process in malaria. Active malarial fever required from ten to seventeen days in contrast to thirty-eight days for artificial fever, and twenty-four and thirty-nine days for typhoid vaccine fever. The paroxysms of malaria were usually of daily occurrence, whereas treatment in the other types of fever was given two or three times each week. The total amount of fever above 100° R. was also greater in malaria, an average of one

hundred twelve hours, in contrast to eighty-seven hours and fifty-five hours for typhoid vaccine fever and artificial fever, respectively. On the other hand, the plasma protein changes of the eight patients given malarial therapy differed only little, regardless of the number and frequency of the paroxysms, the maximum temperature experienced, the total amount of fever above 100° R., or the total duration of malarial therapy. The number of malarial paroxysms (temperature 103° R., and above) ranged from four to thirteen, the total amount of fever above 100° R. from seventy-one to one hundred fifty-four hours, the duration of malaria from fourteen to twenty-two days from the time of inoculation, and that of actual malarial fever from ten to seventeen days. Despite these wide variations the percentage reduction in albumin in the eight patients was nearly the same, namely, from 30 to 40 per cent. One patient after three malarial inoculations experienced only four paroxysms and a total of one hundred ten hours of fever above 100° R. over a twenty-nine-day period, whereas a second patient after one inoculation experienced nine paroxysms and a total of seventy-one hours of fever above 100° R. over a ten-day period. Notwithstanding such differences the reduction in albumin in the former was 34 per cent and in the latter only 41 per cent. Tareev and Gontaewa,[14] however, contrary to our own findings, observed that the reduction in albumin in malarial infections paralleled the clinical severity. They observed more marked reductions in induced malaria than in the naturally acquired form; these were attributed to the more rapid recurrence of fever in the former type.

The rapidly induced anemia is a prominent feature of malaria, and one questions whether this factor plays any part in the low serum albumin levels obtained. Low serum albumin values have been found in anemias, both primary and secondary, but have been attributed to the accompanying malnutrition.[4] These anemias, however, are of a chronic type. The fall in albumin during malaria cannot be attributed to red cell destruction, since both Láng[15] and Bodansky and co-workers[16] found no change in the serum albumin of dogs in which anemia was produced by the administration of phenylhydrazine and acetyl phenylhydrazine, respectively.

The liver has been considered by some as the main source of albumin and the source of much of the globulin.[17] It is the sole source of fibrinogen. It has been shown that infections impair the manufacture of plasma proteins in the liver, despite the fact that protein intake may be maintained.[18] This may well explain the marked reductions in serum albumin (37 and 39 per cent) in two patients who exhibited a "ravenous appetite" throughout malaria yet showed as marked a reduction in albumin as a third patient whose food intake was poorer and whose weight loss was greater (10 pounds in contrast to 4.5 and 2.5 pounds). It does not explain the increases in globulin which were obtained at these times unless one predicates as Loeb[2] has that albumin and globulin are formed by different cells in the liver. Experimental observations reveal that only a small portion of protein is stored in the liver as preformed plasma protein.[17] Thus, not only is the liver unable to synthesize new plasma protein from exogenous material, i.e., food, because of an infection, but it is also unable to convert its stored protein into plasma protein because of a disturbance in the plasma protein-forming mechanism of the liver. The rapid rise in albumin practically immediately after the malaria was terminated, and at a

time when food intake remained essentially unchanged, would tend to verify the importance of infection in impairing serum protein production in the liver. The absence of an infectious process in fever induced by typhoid vaccine and artificial methods may well explain the slight and insignificant fluctuations in albumin which occurred during these types of therapy. In support of this is the finding of a marked reduction in albumin in typhoid fever,[1] though the very limited food intake at this time is an important factor not to be overlooked.

The low serum albumin values of severe infections have been attributed by some to the accompanying malnutrition,[4] i.e., insufficient protein intake. Continuous wasting due to malnutrition causes a steady fall in albumin.[19] It is difficult to evaluate true weight loss in patients undergoing malarial therapy, since subclinical fluid retention may well have occurred during its course because of low albumin values.[6] The weight losses obtained ranged from 1 to 10 pounds and, though they may indicate insufficient total caloric intake, they do not imply an inadequate protein intake. One patient showed a weight gain of 2.5 pounds during malarial therapy at a time when albumin had been reduced 34 per cent, from 4.82 Gm. to 3.23 Gm. No clinical evidence of edema was seen. It is possible that the protein intake of our patients, adequate for the patient when in normal health, was insufficient during malarial fever to supply the increased protein demands when fever was present. It does not appear logical to attribute the rapidly falling albumin during malaria to malnutrition, since the fall in albumin appeared early and even before the malarial fever occurred. This is well shown by the progressive fall in albumin from 4.82 to 4.01 Gm. in one patient over a thirteen-day afebrile incubation period, and the rapid fall in albumin from 5.1 to 4.3 Gm. in a second patient three days after malaria inoculation and one day before fever occurred. Sufficient protein storage in the liver and muscles should be present and readily available at this time to supply the body's needs,[17] especially since the dietary intake of each patient prior to and during the incubation period of malaria had been adequate. The albumin values of the eight patients were within normal limits prior to the induction of the malaria, with the exception of that of one patient who showed a low, unexplained level of 3.67 Gm. Little or no loss of serum albumin occurred in our patients by way of the kidney, since urine examinations infrequently revealed more than a very slight trace of albumin, and kidney function is relatively normal in uncomplicated malaria.[20]

The increases in globulin as a result of therapeutic fever were most marked during malaria, less marked during typhoid vaccine fever, and least marked during artificial fever. An increase in globulin occurs frequently as a result of infections, and is said to parallel the severity of the infection.[5] The increase in globulin during infections has been considered as mainly responsible for the high plasma protein levels found at these times.[21] A close association of certain antibodies with particular globulin fractions has been noted,[2, 4] as is seen by an increase in globulin following the subcutaneous injections of mixed typhoid vaccine. The destruction of red blood cells, as by the production of experimental anemias in dogs, has also resulted in an increase in globulin.[15] The greater increases in globulin during malaria may, therefore, be attributed to the presence of an infection and the destruction of red blood cells. Since

the reticulo-endothelial system participates in the regeneration of globulin,[21] one wonders whether stimulation of this system, said to occur during malaria,[22] may also play some part.

The fibrinogen level during health is relatively constant for any one individual and is not altered by short periods of fasting or by food ingestion.[23] Injury to the liver causes the blood fibrinogen to fall rapidly, the degree of fall paralleling somewhat the severity of liver injury. Regeneration and repair of liver tissue result in a return of the fibrinogen values to normal.[17] Injury to other tissues (as in infections) in the absence of liver injury is followed by an increase in fibrinogen.[17, 23] Fibrinogen values obtained on our patients imply that liver damage occurs as a result of malaria. Values fell considerably, even during the incubation period of malaria, in one patient from 520 mg. to 260 mg. Fibrinogen values obtained on this latter patient during malarial fever remained much below the prefebrile level. The falling values obtained on two additional patients as the malarial fever recurred also point to increasing impairment of liver functions. Clifford has stated that malaria always results in liver damage.[24] Studies of liver function following malarial therapy at this clinic indicate that liver damage of a temporary nature does occur.[25] It is significant that the course of the falling albumin during malaria did not differ in the three patients with probable liver damage from the remaining 5 patients who exhibited no significant reductions in fibrinogen. The rapid rise of albumin following the termination of the malaria would indicate that plasma protein regeneration occurs rapidly after the termination of the infection regardless of the fibrinogen values and probable liver damage. It would also tend to stress the importance of the infectious process per se in interfering with the production of albumin. The increases in fibrinogen as a result of typhoid vaccine fever have been observed by others.[23] It should be stressed, however, that fever per se is probably not the cause of the abnormally increased fibrinogen found in many febrile diseases.[23] Increases in fibrinogen occurred in our patients even during the incubation period of malaria when no fever had occurred. The fall in fibrinogen from 380 mg. to 270 mg. in one patient after eight artificial fever treatments (Chart 3) is difficult to explain. This value was obtained at a time when albumin also had fallen to its lowest level. Though liver function studies have not been carried out in this clinic during artificial fever therapy, occasional instances of jaundice have been observed. Significant pathologic changes in the liver have been found when fever therapy has terminated fatally.

CONCLUSIONS

1. Fever produced by inoculation malaria results in marked changes in the plasma proteins consisting of a rapid and marked drop in albumin, and fluctuations in globulin and fibrinogen. Termination of malarial fever by quinine is followed by a rapid return to normal values.

Fever produced by typhoid vaccine and artificial means (inductotherm) causes only slight fluctuations in the plasma protein fractions.

2. The more marked reduction in albumin during malaria may be due in part to the greater amount and the more rapid recurrence of fever. The in-

fectious process per se present in malaria is probably the most important factor interfering with the synthesis of albumin in the liver even though dietary intake appears adequate.

3. The increases in globulin are greater during malaria than during fever induced by typhoid vaccine or artificial means. The presence of an infectious process and the destruction of red blood cells during malaria account for the greater increases in globulin.

4. Fibrinogen is increased during malaria and typhoid vaccine fever. The progressive fall in fibrinogen, which occasionally occurs during malaria, indicates probable liver damage.

REFERENCES

1. Lloyd, R. B., and Paul, S. N.: Serum Protein Changes in Malaria and Typhoid Fever With Suggestions as to Their Possible Bearing on Immunity, Indian J. M. Research **17**: 583, 1929.
2. Loeb, R. F.: Medical Progress; Plasma Proteins in Health and Disease, New England J. Med. **224**: 980, 1941.
3. Madden, S. C., Winslow, P. M., Howland, J. W., and Whipple, G. H.: Blood Plasma Protein Regeneration as Influenced by Infection, Digestive Disturbances, Thyroid and Food Proteins. A Deficiency State Related to Protein Depletion, J. Exper. Med. **65**: 431, 1937.
4. Peters, J. P., and Eisenman, A. J.: Serum Proteins in Disease Not Primarily Affecting the Cardiovascular System or Kidneys, Am. J. M. Sc. **186**: 808, 1933.
5. Wiener, H. J., and Wiener, R. E.: Plasma Proteins, Arch. Int. Med. **46**: 236, 1930.
6. Kopp, I., and Solomon, H. C.: The Relationship of Hypoalbuminemia to the Edema of Malaria, Am. J. M. Sc. **202**: 861, 1941.
7. Judd, H. S.: Some of the Problems of Malarial Treatment of Neurosyphilis, Washington Bull. **2**: 8, 1938.
8. Howe, P. E.: The Determination of Proteins in Blood. A Micro-Method, J. Biol. Chem. **49**: 109, 1921.
9. Folin, O., and Wu, H.: System of Blood Analysis, J. Biol. Chem. **38**: 81, 1919.
10. Howe, P. E.: The Use of Sodium Sulphate as the Globulin Precipitant in the Determination of Proteins in Blood, J. Biol. Chem. **49**: 93, 1921.
11. Cullen, E. G., and Van Slyke, D. D.: Determination of Fibrin, Globulin, and Albumin Nitrogen of Blood Plasma, J. Biol. Chem. **41**: 587, 1920.
12. Villela, G. G., and de Castro Teixeira, J.: Plasma Proteins in Hookworm Disease, Mem. Inst. Oswaldo Cruz **23**: 41, 1930.
13. Eichelberger, L., and McCluskey, K. L.: Chemical Studies in Tuberculosis; Plasma Proteins, Cholesterol, and Corpuscle Volume, Arch. Int. Med. **40**: 831, 1927.
14. Tareev, E. M., and Gontaewa, A. A.: Zur Bestimmung des Albumin-Globulin-Mischungsverhältnisses im Blutserum bei Malaria, Arch. f. Schiffs- u. Tropen-Hyg. **37**: 28, 1933.
15. Láng, S.: Über die Wirkung des Phenyl-hydrazins auf die Bluteiweiss-körper, Ztschr. f. d. ges. exper. Med. **80**: 637, 1932.
16. Bodansky, M., Morse, S. W., Kiech, V. C., and Bramkamp, R. B.: The Distribution of Protein in the Blood in Experimental Anemia, J. Biol. Chem. **74**: 463, 1927.
17. Madden, S. C., and Whipple, G. H.: Plasma Proteins; Their Source, Production and Utilization, Physiol. Rev. **20**: 194, 1940.
18. Madden, S. C., Finch, C. A., Swalbach, W. G., and Whipple, G. H.: Blood Plasma Protein Production and Utilization; Influence of Amino Acids and of Sterile Abscesses, J. Exper. Med. **71**: 283, 1940.
19. Bodansky, M.: Introduction of Physiological Chemistry, New York, 1938, John Wiley and Sons, Inc., p. 240.
20. Furuichi, T.: Quoted by Rudolf, G. De M.: Therapeutic Malaria, London, 1927, Humphrey Milford, p. 150.
21. Whipple, G.: Protein Production and Exchange in the Body, Including Hemoglobin, Plasma Protein and Cell Protein (Mellon Lecture), Am. J. M. Sc. **196**: 609, 1938.
22. Taliaferro, W. H., and Mulligan, H. W.: Histopathology of Malaria With Special Reference to Function and Origin of Macrophages in Defence, Indian M. Research Mem. No. 29, 1, 1937.
23. Ham, T. H., and Curtis, F. C.: Plasma Fibrinogen Response in Man, Medicine **17**: 413, 1938.
24. Clifford, H.: Quoted by Wile, U. J., and Sams, W. M.: Study of Jaundice in Syphilis; Its Relation to Therapy, Am. J. M. Sc. **187**: 297, 1934.
25. Unpublished data.

LABORATORY METHODS

GENERAL

"ELECTROCARDIOGRAPHIC ANAGRAMS"*

A Method of Teaching Electrocardiography

Joseph E. F. Riseman, M.D., Boston, Mass.

THE model described in this report has been used with considerable success in teaching electrocardiography in a simple logical manner. In principle it consists of cards of uniform sizes on which portions of P, Q, R, S, and T complexes are drawn to scale in such a fashion that the cards may be joined together or superimposed one upon another to form any of the more common electrocardiographic patterns. If only one portion of the electrocardiogram is changed at one time, the student soon learns to recognize the abnormality and to interpret its significance in a logical fashion. This method eliminates the necessity of textbook descriptions or patterns; it enables the student to lose some of his fear of electrocardiograms; and it allows him to test himself by creating characteristic patterns.

DIRECTIONS FOR PREPARING MODEL

Models of any size can be made. For classroom work, electrocardiographic complexes drawn to a scale of ½ inch for each millimeter of amplitude or each 0.4 seconds of duration have been found most serviceable; these are drawn on cards 10 inches high and 3, 6, or 12 inches wide. A complete set consists of 90 cards; 16 cards, 10 by 12 inches; 14 cards, 10 by 6 inches; and 60 cards, 10 by 3 inches.

Materials.—The following materials are necessary; 38 pieces of heavy white noncurling cardboard, each measuring 10 by 12 inches; two sheets of transparent cellulose acetate, approximately 10 by 12 inches; ruler; pencil; soft wax pencil; black poster paint; drawing pen; camel's hair paint brush; scissors; sharp knife or razor blade.

Method.—With a pencil draw two lines, 1 inch apart and 12 inches long, through the center of each card; this represents the electrocardiographic base line, or a string shadow 2 mm. wide. On four of these cards this base line is inked in. Two cards are left intact (Fig. 1 *a*). One card is cut in two to form two models, 10 by 6 inches each (Fig. 1 *b*); one is cut to form four cards, 10 by 3 inches (Fig. 1 *c*). A black ink dot is placed in the upper left corner of each of these and subsequent cards to make possible rapid recognition of the upright position.

*From the Medical Research Department, Beth Israel Hospital, and the Department of Medicine, Harvard Medical School, Boston.

Received for publication, August 25, 1941.

Normal Complexes (Fig. 2).—Starting ½ inch from the left-hand margin of one card, draw a P-wave, one inch high and approximately 1½ inches wide. Beginning 3 inches from the left-hand margin, draw a QRS complex, 1.25 inches in width, the Q-wave should be approximately ½ inch in amplitude, the R- and S-waves 3 to 4 inches in amplitude. Beginning 6 inches from the left-hand margin, a T-wave is drawn ¾ inch in amplitude and 2 inches wide; if desired the terminal limb of the T-wave can be drawn to dip ¼ inch below the base line, returning to the isoelectric level, 9 inches from the left-hand margin. By following these directions the P-wave will be the upper limit of normal in amplitude; the T-wave will be the lower limit of normal in amplitude; the QRS complex and the PR-interval will be the upper limit of normal in duration (Fig. 2 *a*). One piece of cellulose acetate is superimposed on this drawing; the contour of the

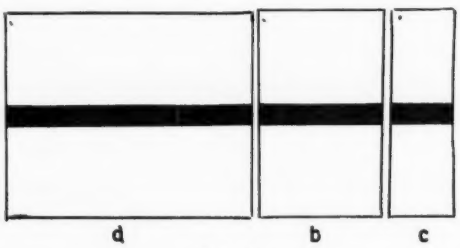

Fig. 1.

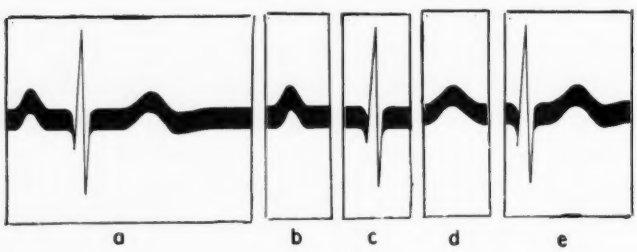

Fig. 2.

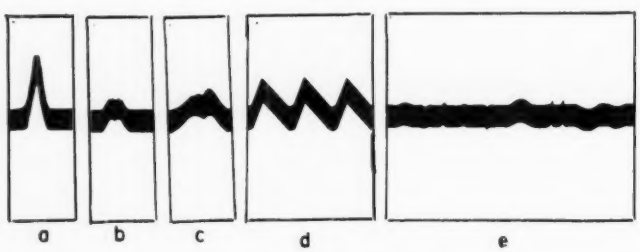

Fig. 3.

diagrammatic complex is traced with a wax pencil, and the resultant P-QRS-T-complex is cut out. This results in a templet, which can be used to draw normal complexes of identical characteristics. A total of six P-QRS-T-complexes are prepared from this templet, and the outlines are filled in with poster paint.

Lines are drawn on four cards to divide them into sections, 10 by 3 inches. By using the appropriate portions of this same templet, six P-waves (Fig. 2 *b*) four QRS complexes (Fig. 2 *c*) and six T-waves (Fig. 2 *d*) are drawn on 10 by

3 inch cards. With the same templet normal QRS-T complexes are drawn on four 10 by 6 inch cards to depict ST-segments of short duration (Fig. 2 *e*).

Abnormal P-Waves.—Six P-waves, 2 inches high (Fig. 3 *a*), and six notched P-waves (Fig. 3 *b*), ¾ inch high, are drawn on 10 by 3 inch cards after preparing the appropriate templets. Four cards (10 by 3 inches) are prepared showing normal T-waves on which abnormal P-waves are partially superimposed (Fig. 3 *c*).

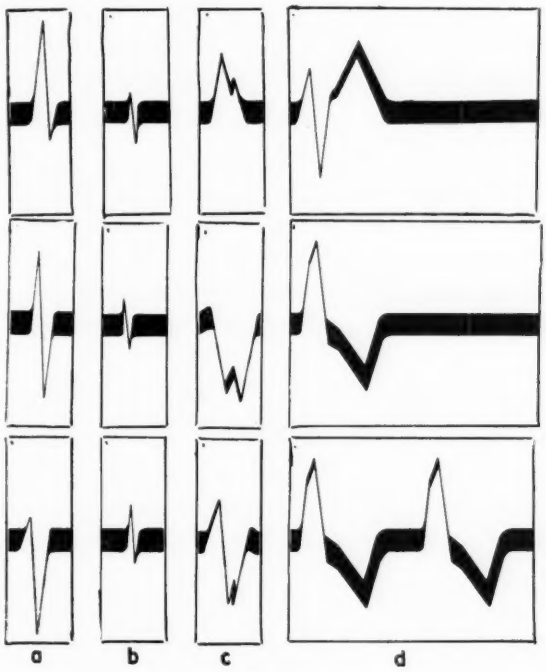

Fig. 4.

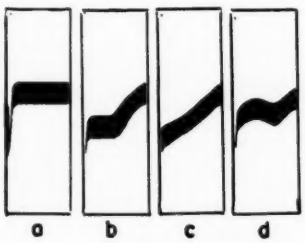

Fig. 5.

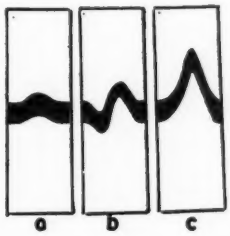

Fig. 6.

A flutter wave, 2 inches in duration, is drawn, and from the templet four cards, 10 by 6 inches, are prepared, each bearing three flutter waves (Fig. 3 *d*). By making the base line of three cards irregular, the f waves of auricular fibrillation are depicted (Fig. 3 *e*).

Abnormal QRS Complexes.—QRS complexes to show axis deviation are drawn on three cards (Fig. 4 *a*), 10 by 3 inches, as follows: On one card a large R-wave and a small S-wave are drawn, and a templet is made. By inverting and reversing the templet, a complex with a small R-wave and a large S-wave is

drawn on a second card. The third complex is drawn to represent an average of these two types of QRS complexes.

QRS complexes of low amplitude are demonstrated (Fig. 4 *b*) on three 10 by 3 cards. Each card represents one of the three standard leads. The complexes are drawn less than 1¼ inches in width and less than 3½ inches in amplitude.

Bundle branch block (spreading, slurring, and notching) is represented on three 10 by 3 inch cards (Fig. 4 *c*); the major deflection is upright on one card, and downward in another card; the third card represents an average of the first two.

Premature ventricular contractions of two different designs are drawn on 10 by 3 inch cards (Fig. 4 *d*). The abnormal QRS-T complexes should begin at the left-hand margin of the card and occupy 4 to 5 inches; the remainder of the card serves to depict the compensatory pause. Using a templet drawn from one of these extrasystoles, three additional cards, each containing two ventricular premature beats, are drawn so that when these are joined together they illustrate a run of ventricular extrasystoles or ventricular tachycardia.

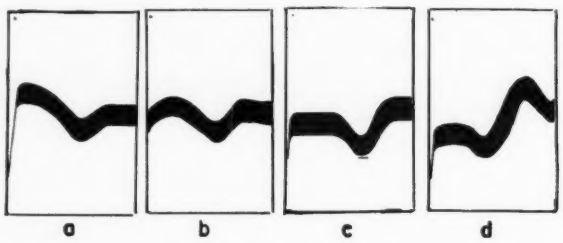

Fig. 7.

Abnormal ST-Segments and T-waves.—Four types of abnormal ST-segments are illustrated on 10 by 3 inch cards (Fig. 5). These are drawn so that the left-hand margin of each can be superimposed on the normal S-wave, and the right-hand margin can be superimposed on the normal T-wave. One card depicts an elevated ST-segment parallel to, and 1 inch above, the base line (Fig. 5 *a*). A second card depicts a depressed ST-segment, also parallel to the base line (Fig. 5 *b*). A third card begins 1 inch below the base line, and then runs diagonally up to reach the peak of the T-wave (Fig. 5 *c*). The fourth card illustrates coving (Fig. 5 *d*). Four T-waves, ½ inch high, are drawn on cards 10 by 3 inches (Fig. 6 *a*). One diphasic T-wave (Fig. 6 *b*) is drawn on another 10 by 3 inch card. Six T-waves, 3 inches in amplitude (Fig. 6 *c*), are drawn on 10 by 3 inch cards; these may be used to depict normal T-waves of high amplitude or T-waves on which P-waves have been superimposed.

Four 10 by 6 inch cards, illustrating combinations of abnormal ST-segments and T-waves, are drawn. One depicts an elevated ST-segment and inverted T-wave (Fig. 7 *a*); another, coving with an inverted T-wave (Fig. 7 *b*); a third, a depressed ST-segment, with an inverted T-wave (Fig. 7 *c*); and a fourth, a depressed ST-segment with diphasic T-wave (Fig. 7 *d;* digitalis T-waves).

To demonstrate these cards a stand can be made according to Fig. 8. This stand also serves as a container for the cards when they are not in use.

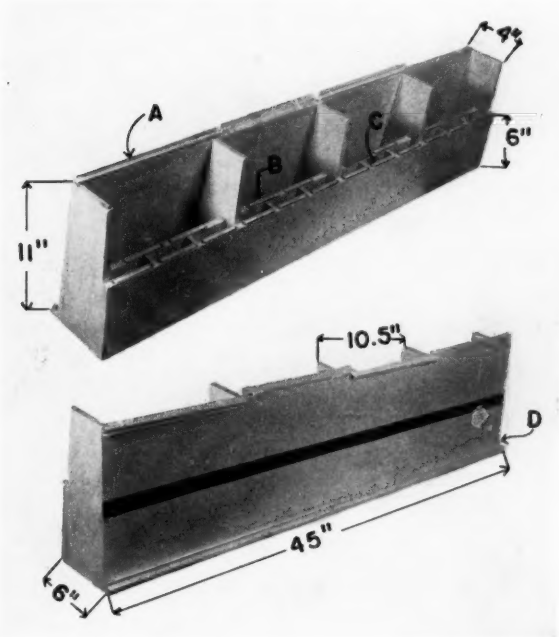

Fig. 8.—Viewing stand for demonstrating electrocardiographic patterns. (This viewing stand can be made of ½ inch stock.) *A*, Hinged molding to hold upper edge of cards in place; *B*, container for large cards, 10½ by 2½ inches; *C*, container for small cards, 3⅛ by 1½ inches; and *D*, grooved molding to hold lower edge of cards in place.

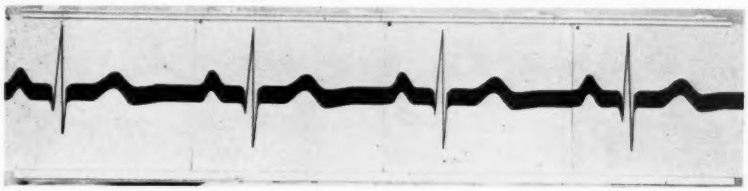

Fig. 9.

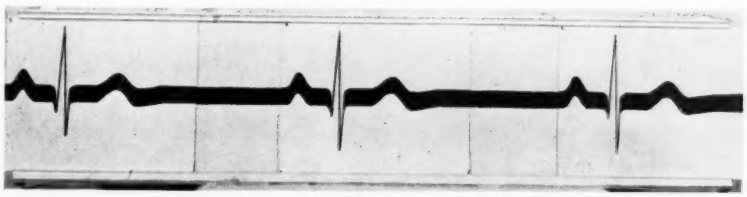

Fig. 10.

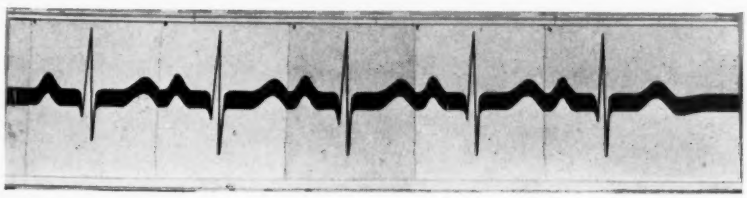

Fig. 11.

METHOD OF USE

In use, normal sinus rhythm is first illustrated by joining end-to-end four normal P-QRS-T complexes (Fig. 9). Arrhythmias arising from the sino-auricular node are then illustrated: sinus bradycardia, by separating three normal P-QRS-T complexes (Fig. 10); sinus tachycardia, by partially superimposing the complexes so that five occupy the same horizontal area (Fig. 11).

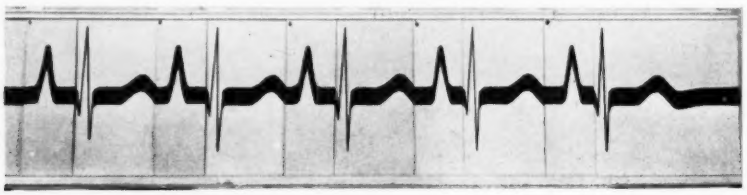

Fig. 12.

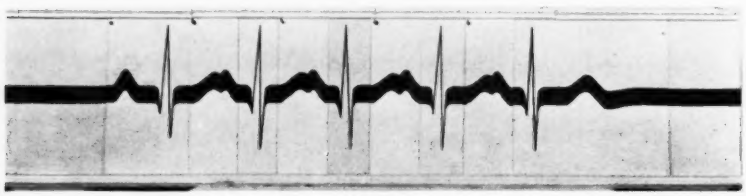

Fig. 13.

Abnormalities of the P-waves are next illustrated. Auricular tachycardia is depicted by superimposing an abnormal P-wave on each of the normal P-waves of the sinus tachycardia (Fig. 12), or by further superimposition of the normal complexes so that the P-waves cover the T-waves, and then superimposing the combined P-T waves (Fig. 13), large T-waves, or inverted P-waves. In a similar fashion abnormalities of the PR-interval (varying degrees of block) are illustrated, then abnormalities of the QRS complexes, the ST-segment, and the T-waves. Practically all the electrocardiographic patterns, which one encounters clinically, can be created by this method.

SUMMARY

The model described consists of units of electrocardiographic complexes prepared in such a fashion that they can be joined together so as to illustrate diagrammatically most of the common electrocardiographic patterns. The model has proved to be of considerable value in teaching beginners how to interpret electrocardiograms.

SIMPLE METHOD OF EVALUATING BLOOD PLATELETS*

EVANS W. PERNOKIS, M.D., CHICAGO, ILL.

THE procedure for accurately seeing, identifying, and counting the blood platelets has become more important with the discovery that splenectomy cures a high percentage of patients afflicted with idiopathic purpura hemorrhagica. The methods used and recommended by most textbooks are based on dilution and counting with the hemacytometer. The most popular diluting fluid is that of Wright and Kinnicutt, though the fluids recommended by Rees and Ecker, and Guy and Leake are also extensively used. The Fonio method, so highly recommended by the German school, which uses the magnesium sulfate as a diluent before spreading the blood on the slide for a smear, also has been extensively used. After several years of trial with the dilution methods and the Fonio method with students in classes of Laboratory Diagnosis, it is our opinion that the following method is the simplest and least time-consuming for the accurate identification and counting of blood platelets.

The solution used is a 1 per cent brilliant cresyl blue in absolute alcohol. A drop of the solution is placed on the edge of a slide (thoroughly cleaned and flamed), and spread out in a manner similar to spreading blood for a blood smear. The solution evaporates readily, leaving a thin coat of stain. An ear puncture is made, and a drop of blood the size of a black pinhead is placed on a thoroughly cleaned cover slip and overlaid on the stained slide. A red blood cell count is also drawn at the same time. The size of the drop must not be too large so that it spreads out uniformly filling the cover slip area with a thin film of blood. The platelets take the brilliant cresyl blue stain and appear as pale blue bodies floating between the less colored red blood cells. Variations in shape and size are also readily noted in the preparation. A total of 1,000 red blood cells are counted on the film, and the number of platelets in the same area is noted. From the value of the red blood count, the platelet count is calculated. For example, if in counting 1,000 red blood cells, we encounter 75 platelets, and the patient's blood count is 4,750,000 red cells, by multiplying the first four figures of the red count with the number of platelets in 1,000 red cells we get the platelet count, which in the above case would be 75 times 4,750, or 356,250 platelets.

This method offers the advantage that reticulocytes may also be counted on the same preparation. If kept in a Petri dish lined with moistened filter paper, it may be kept several hours without the platelets disintegrating. After first observing the moist preparation, the cover slip may be removed with light pressure and counterstained with Wright's stain or May-Grünwald-Giemsa for further observation of the platelets and a differential count.

*From the Presbyterian Hospital, Chicago.
Received for publication, September 29, 1941.

A SHAKING DEVICE USED IN THE COLLECTION OF BLOOD FOR TRANSFUSION*

L. W. Diggs, M.D., and H. B. Turner
Memphis, Tenn.

A SIMPLE, noiseless and relatively inexpensive shaking device (Fig. 1) has been developed in the Blood Bank at the John Gaston Hospital. The power is supplied by a small electric motor from a portable victrola. The back and forth motion is obtained by a shaft attached to an eccentric disk. The carriage for the collecting bottle is swung from two metal supports 12 inches in length.

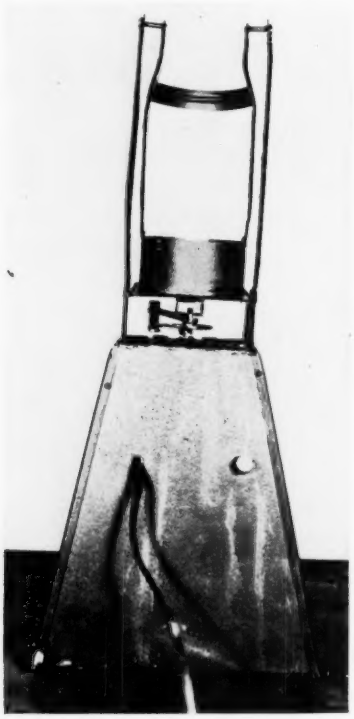

Fig. 1.—A shaking device used in the collection of blood for transfusion.

The fulcrum is placed at the level of the top of the bottle so that motion and vibration at this point are minimal. The carriage is open at the top so that the collecting bottles can be easily inserted and removed. The height of the instrument is 23 inches, the width at the base 10 inches, and the width at the top 5 inches.

*From the Division of Medicine and the Clinical Laboratories of the John Gaston Hospital and the University of Tennessee, Memphis.
Received for publication, November 3, 1941.

The shaking device has been used for the collection of approximately 2,000 flasks of blood for transfusions and has proved to be entirely satisfactory. The assistant is spared the tiresome and awkward task of keeping the flask of blood in motion during the three to fifteen minutes required to collect 500 c.c. of

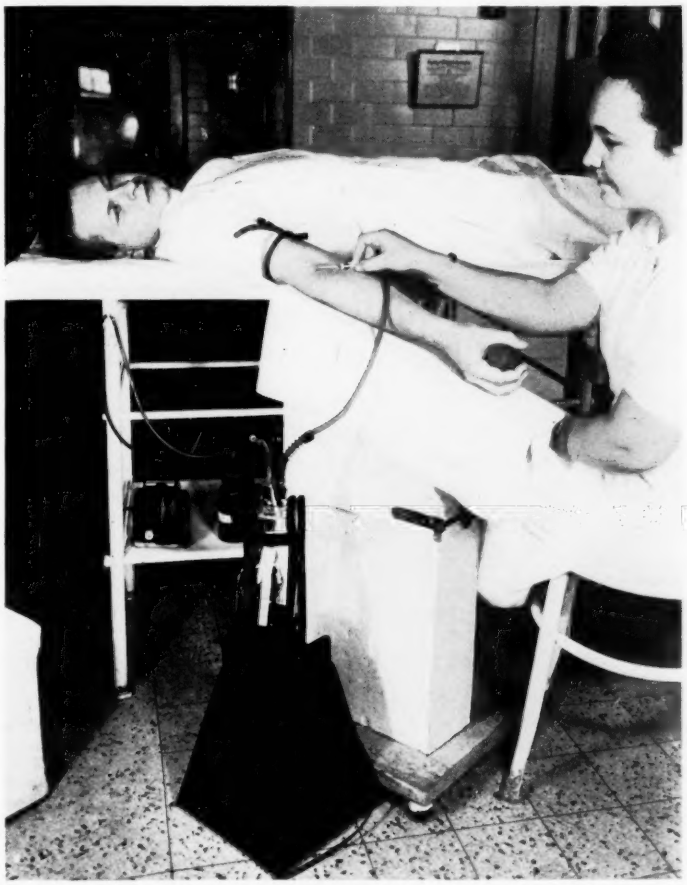

Fig. 2.—The collection of blood from a donor by the closed citrate method using "the shaker."

blood. With the shaker it is possible for one person to collect the blood unassisted. The gentle agitation furnished by this instrument is adequate to prevent coagulation. In our routine collections the blood is simply allowed to run downhill without the use of negative pressure, but if negative pressure is desired, the suction pump may be connected as in Fig. 2.

The instrument was built by the students at the William R. Moore School of Technology under the direction of Mr. J. M. Smith.

THE RELATIONSHIP OF THE PLASMA PROTEINS TO THE CORRECTED SEDIMENTATION RATE*

ISRAEL KOPP, M.D., BOSTON, MASS.

MANY observers have demonstrated a suggestive relationship between the red blood cell sedimentation rate and the fibrinogen content of the plasma, and some have concluded that the fibrinogen content determines the sedimentation rate.[1, 2] Others,[3, 4] however, have failed to observe any such causal relationship and have found no correlation between the sedimentation rate and any of the plasma proteins. Marked changes in the sedimentation rate occur even without any changes in the plasma proteins.[4] Increases in globulin and extreme degrees of lipemia are also known to affect the sedimentation rate.[5]

Repeated determinations of the plasma protein fractions and the sedimentation rate on patients undergoing therapeutic fever for central nervous system syphilis have afforded an opportunity to review again the problem of the relationship if any between the sedimentation rate and the plasma proteins.

MATERIAL AND METHODS

Five male patients with general paresis, ranging from 39 to 52 years in age, were hospitalized for fever therapy. The five patients were given the following therapy: tertian malaria to two patients; tertian and quartan malaria to one patient; tertian malaria and typhoid vaccine fever to one patient; artificial fever and malaria to the fifth patient.

Venous blood samples were obtained without stasis and with the patient in a fasting state. In most instances the patient was afebrile. Potassium oxalate, 2 mg. per cubic centimeter of blood, was used as the anticoagulant for the determination of the plasma proteins.

The total protein was obtained by the determination of the total nitrogen by a modified micro-Kjeldahl[6]; the nonprotein nitrogen by the method of Folin and Wu[7]; the albumin and globulin by the sodium sulfate method of Howe[8]; and the fibrinogen by precipitation as fibrin by the method of Cullen and Van Slyke.[9] In the last series of studies the fibrinogen was precipitated as above,[9] and the nitrogen content of the fibrin was determined directly.

The sedimentation rate of whole blood was determined by the method of Rourke and Ernstene[10] and corrected for the hematocrit readings by their correction chart. Dry oxalate mixture, 6 mg. of crystalline ammonium oxalate and 4 mg. of crystalline potassium oxalate for 5 c.c. of blood, was used as the anticoagulant.

*From the Boston Psychopathic Hospital.
Received for publication, November 21, 1941.

The sedimentation rate of defibrinated blood was determined by the method of Ham and Curtis.[2] Fibrinogen was removed by rotating 5 c.c. of blood together with 12 small glass beads in an Erlenmeyer flask for from ten to fifteen minutes, and then filtering through two layers of gauze. The defibrinated blood was then mixed, and the sedimentation rate was determined as with whole blood. Because of the slow settling, readings were made over a period of three hours. Corrections were made for the hematocrit according to the charts of Ham and Curtis,[2] and Rourke and Ernstene.[10]

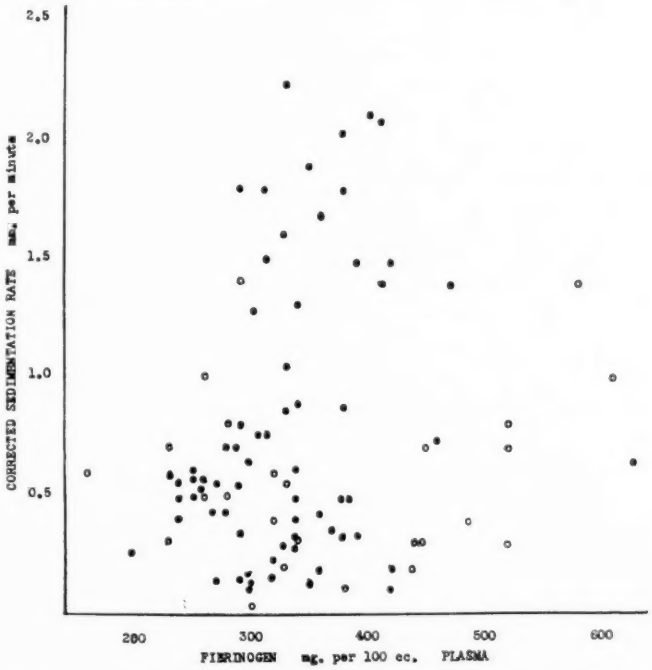

Chart 1.—○ Fibrinogen by difference, • Fibrinogen by direct method.

RESULTS

Ninety-two sedimentation rates were obtained during malaria, typhoid vaccine, and artificial fever therapy. Taken as a whole no linear relationship could be found between the corrected sedimentation rates and the fibrinogen values (Chart 1). The sedimentation rate varied more than 1,500 per cent for the same fibrinogen value. Fibrinogen values also varied widely for similar sedimentation rates, as for example, sedimentation rates of 0.60 mm. and 0.65 mm. per minute for fibrinogen values of 160 mg. and 630 mg., respectively. The sedimentation rates also bore no absolute relationship to albumin, albumin-globulin, or globulin-fibrinogen ratios.

It was observed frequently that when the more rapid sedimentation rates could not be explained on the basis of fibrinogen values alone, the accompanying globulin values were close to the upper limit of normal, 3 Gm. per 100 c.c. or above. However, wide variations in the sedimentation rate, as much as 2,200 per cent, occurred even for similar globulin values (Chart 2).

The sedimentation rates of each patient revealed a suggestive linear relationship with fibrinogen values in only two of the five patients, one during malarial therapy and the second during artificial fever therapy. Changes in the globulin fraction during treatment appeared less marked in the two patients than in the others. The subsequent administration of tertian malaria to the patient who had received artificial fever therapy resulted in more scattered sedimentation rates for nearly similar fibrinogen values (Chart 3). Globulin values, though higher at this time than during artificial fever therapy, were still within normal limits.

An attempt was made to evaluate the importance of globulin in the determination of the sedimentation rate by obtaining 54 defibrinated blood sedimentation rates on three patients during malarial therapy, and on one of this group during a course of typhoid vaccine fever. The removal of fibrinogen did not prevent an increase in the sedimentation rate, but the increases when present were less marked than those of whole blood, i.e., fibrinogen was not removed. It will be observed (Chart 2) that the sedimentation rates with and without the presence of fibrinogen follow about the same pattern when compared with the globulin values. No correlation was found between the defibrinated sedimentation rates and globulin values when the latter were 2.8 Gm. per 100 c.c. or less. Above this level, however, a suggestive linear correlation was present, the defibrinated sedimentation rates showing marked increases with only slight increases in globulin.

DISCUSSION

Our findings when taken as a whole reveal little correlation between the corrected whole blood sedimentation rate and fibrinogen values in therapeutic malaria and typhoid vaccine fever. The corrected sedimentation rates obtained during artificial fever therapy of one patient and malaria therapy of a second patient bore a suggestive linear relationship to fibrinogen values. Changes in the fibrinogen, globulin, and red blood cells are quite marked during malaria, less marked during typhoid vaccine fever, and least marked during artificial fever. It is probable that a better relationship between the sedimentation rate and fibrinogen may exist when changes in the plasma proteins and red blood cells are not rapid or marked. Ropes and co-workers,[4] however, have observed marked changes in the sedimentation rate in both acute and chronic infections even without any change in the concentration of the plasma proteins. No absolute correlation was found by these workers between the sedimentation rate and any of the plasma protein fractions.

The sedimentation rates obtained with whole blood and defibrinated blood samples show the importance of globulin. Regardless of fibrinogen levels, globulin values of 2.9 Gm. per 100 c.c. and above were frequently accompanied by rather marked increases in the sedimentation rates of whole blood but less pronounced in the defibrinated samples. Defibrinated sedimentation rates with globulin values less than 2.9 Gm. per 100 c.c. ranged mostly from 0.01 to 0.2 mm. per minute (Chart 2) and were frequently above the normal range of 0.01 to 0.03 mm. per minute.[2] Defibrinated sedimentation rates increased consider-

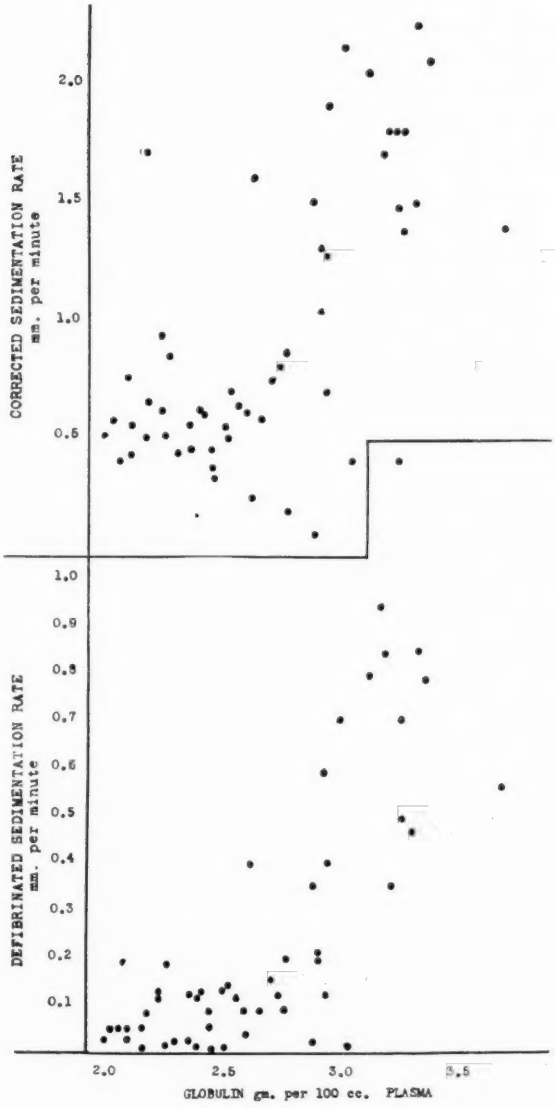

Chart 2.

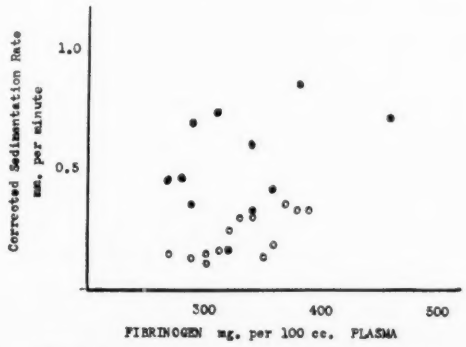

Chart 3.—• Malaria. ○ Artificial fever.

ably up to 0.95 mm. per minute, when globulin levels exceeded 2.9 Gm. per 100 c.c. Since it has been shown that albumin does not increase the sedimentation rate, but may even slow it,[4] the increases in the defibrinated sedimentation rate must be attributed to changes in the globulin fraction, quantitatively or qualitatively, or to some other unknown factor. Ham and Curtis[2] have also noted marked elevations in the defibrinated sedimentation rate when serum globulin was abnormally increased. They have found, too, a rough correlation to exist between the concentration of serum globulin when above 3 Gm. per 100 c.c. and the defibrinated sedimentation rate provided lipemia is not present.

Other factors which may affect the sedimentation rate and account for its lack of correlation with the fibrinogen values obtained on our patients are the following: the mean corpuscular volume of the red blood cells, the presence of a lipemia, and liver pathology.

Changes in the mean corpuscular volume of the red blood cells are known to affect the sedimentation rate and are not taken into account when the corrected sedimentation rate is determined.[2] The mean corpuscular volume of the blood during therapeutic fever showed fluctuations as great as –12 to +17 cubic microns; these were especially marked during malarial therapy. The presence of a lipemia increases the sedimentation rate, but determinations on a number of our own patients and observations of others[11] reveal a reduction and not an increase of the cholesterol content of the blood in malaria. We do not know whether changes in the cholesterol occur during fever induced by typhoid vaccine or by artificial means. Gilligan and Ernstene[1] have stated that in liver diseases factors other than fibrinogen, among them globulin, may play an important role in the determination of the sedimentation rate. They, and others,[2] have observed a rapid sedimentation rate in acute hepatitis and cirrhosis of the liver. It is the feeling of Clifford[12] that the liver is damaged in malaria and liver function tests carried out in this clinic show occasional but transient liver damage. In addition progressive reductions in fibrinogen have been observed during therapeutic malaria which would tend to corroborate this. However, rapid sedimentation rates have been obtained on our patients even when fibrinogen has fallen to the lower limit of normal (190 mg. per 100 c.c. of blood) and globulin has remained within normal limits. This does not eliminate the possibility of qualitative changes in the globulin fraction.

An attempt has been made by Ropes and co-workers[4] to explain the inconsistencies between sedimentation rates and fibrinogen values. These workers attribute the variations in the sedimentation rate to modifications in the physical state of the plasma colloids with consequent changes in the electric charges on the proteins and red blood cells. Changes in the colloidal state and increases in the sedimentation rate may thus occur without any change in fibrinogen or globulin values.

CONCLUSIONS

1. Corrected whole blood sedimentation rates and plasma protein values were determined repeatedly on five patients undergoing therapeutic fever (malaria, typhoid vaccine, and artificial fever) for general paresis.

2. No absolute correlation could be found between fibrinogen, albumin, albumin-globulin, or globulin-fibrinogen ratios on the one hand, and the corrected sedimentation rate on the other.

3. The corrected sedimentation rate was increased in most instances when globulin values rose to the upper limit of normal (3 Gm. per 100 c.c.) and above, regardless of fibrinogen levels.

4. Other factors which may influence the sedimentation rate during therapeutic fever are changes in the mean corpuscular volume of the red blood cells and products of probable liver damage.

REFERENCES

1. Gilligan, D. R., and Ernstene, A. C.: The Relationship Between the Erythrocyte Sedimentation Rate and the Fibrinogen Content of the Plasma, Am. J. M. Sc. **187**: 552, 1934.
2. Ham, H. T., and Curtis, F. C.: Sedimentation Rate of Erythrocytes. Influence of Technical, Erythrocyte and Plasma Factors and Quantitative Comparison of Five Commonly Used Sedimentation Methods, Medicine **17**: 447, 1938.
3. Greisheimer, E. M., Johnson, O. H., and Ryan, M.: The Relationship Between Sedimentation Index and Fibrin Content in Relatively Normal Individuals, Am. J. M. Sc. **177**: 816, 1929.
4. Ropes, M. W., Rossmeisl, E., and Bauer, W.: Relationship Between Erythrocyte Sedimentation Rate and Plasma Proteins, J. Clin. Investigation **18**: 791, 1939.
5. Gilligan, D. R.: The Rate of Sedimentation of the Erythrocytes, Cyclopedia Medicine Surgery and Specialties, Philadelphia, 1939, F. A. Davis Company, p. 669.
6. Howe, P. E.: The Determination of Proteins in Blood, a Micro Method, J. Biol. Chem. **49**: 109, 1921.
7. Folin, O., and Wu, H.: A System of Blood Analysis, J. Biol. Chem. **38**: 81, 1919.
8. Howe, P. E.: The Use of Sodium Sulphate as the Globulin Precipitant in the Determination of Proteins in Blood, J. Biol. Chem. **49**: 93, 1921.
9. Cullen, G. E., and Van Slyke, D. D.: Determination of the Fibrin, Globulin, and Albumin Nitrogen of Blood Plasma, J. Biol. Chem. **41**: 587, 1920.
10. Rourke, M. D., and Ernstene, A. C.: Method for Correcting Erythrocyte Sedimentation Rate for Variations in Cell Volume Percentage of Blood, J. Clin. Investigation **8**: 545, 1930.
11. Borel, R., Pons, E., Advier, M., and Guillerm, J.: Cholesterinémie et glycémie dans le paludisme en cochinchine, Ann. Inst. Pasteur **40**: 152, 1926.
12. Clifford, H.: Quoted by Wile, U. J., and Sams, W. M.: Study of Jaundice in Syphilis; Its Relation to Therapy, Am. J. M. Sc. **187**: 297, 1934.

DEHYDRATION IN EMBALMED SPECIMENS EXPOSED TO AIR

F. Lloyd Hansen, M.A., and N. C. Pervier, Ph.D.
Minneapolis, Minn.

ONE of the claims frequently made for glycerin and other polyhydric alcohols as embalming fluid ingredients, is that they retard the loss of water from the subject. We have made a study of this claim from both theoretical and practical viewpoints.

A fresh specimen or body may be regarded as a hydrophylic colloidal system, containing about 60 per cent water. This water content is composed of two components: free water, which provides the proper life environment, and bound water, that which is bound to the biocolloids of tissue through coordinate valence linkages. About 25 per cent of the total water may be in the latter form.[1] Such bound water is less susceptible to evaporation loss than the free water. Moreover, as a result of incipient putrefaction, the complex colloidal structures become degraded and begin to lose the power of binding water. Formaldehyde fixation causes profound changes in protein structures, converting them into insoluble inert products with little water-binding power. As a result of both of these processes, it is logical to regard most of the water found in dead animal bodies as free water. As such, the water is as free to evaporate as is the water in an open dish. The prevention of this loss of water is important in the preservation of museum specimens and bodies which must be preserved for a long time. Water loss from bodies placed in sealed glass top caskets presents a specially troublesome problem.

An obvious method of reducing the evaporation is to dissolve additional solutes in the injection fluid. All nonvolatile solutes reduce the vapor pressures of solutions, in accordance with the law of Raoult. Those which are hygroscopic and form hydrates are most efficient as evaporation retarders. In the tobacco industry[2] glycerin, ethylene glycol, and diethylene glycol have been successfully added in order to maintain the moisture content of tobacco under storage. It has seemed natural to expect that these compounds would also prevent the drying out of animal remains even though larger concentrations of water occur in them.

An inspection of the vapor pressure curves of solutions of these compounds shows that this expectation cannot be realized to any great extent. In the case of glycerin (Fig. 1) a solution must have a concentration of 80 per cent by weight in order to remain in equilibrium with air having a relative humidity of 50 per cent. If the relative humidity is even 95 per cent, so that evaporation is greatly retarded, the solution must still contain 33 per cent of pure glycerin. Thus a normal adult body would need to have injected into it over 2.5 gallons

Received for publication, December 4, 1941.

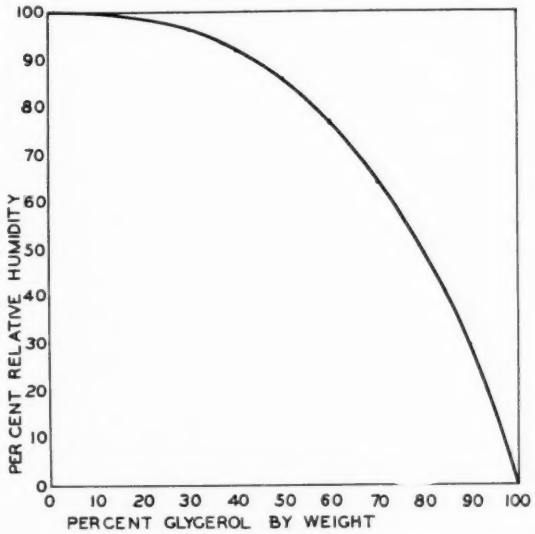

Fig. 1.—Relative humidity over aqueous glycerol solutions.

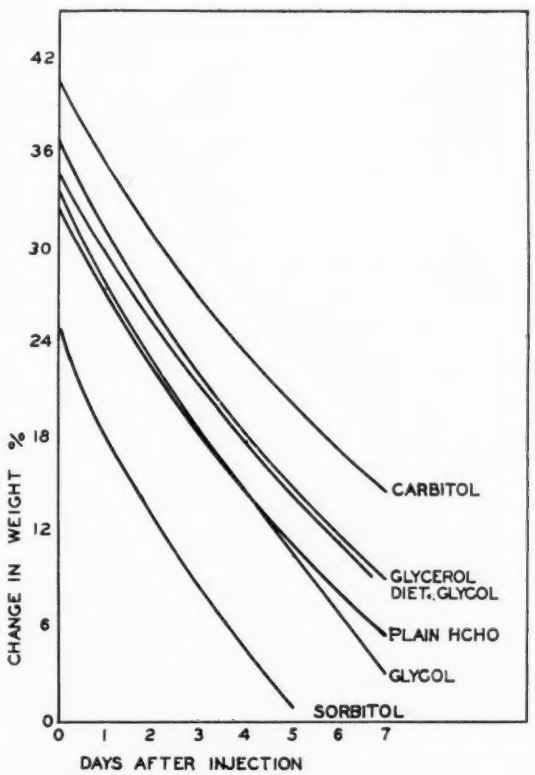

Fig. 2.—Water loss from arterially injected specimens.

of pure glycerin in order to prevent water loss from occurring upon exposure to air at 95 per cent relative humidity. A greater amount of another compound is required if its molecular weight exceeds that of glycerin. In the case of glycol, which is the only nonvolatile alcohol having a molecular weight less than that of glycerin, about two gallons would be required. It is obviously impossible to inject these huge amounts of viscous liquids and we may, therefore, conclude that it is impossible to prevent the gradual loss of water from a body by adding these compounds to the fluid used in injecting it. None of the commercial fluids we examined privately contained over 6 per cent of glycerin or similar product when diluted according to directions, and it is evident that these would have only a slight effect in delaying the loss of water from a body.

EXPERIMENTAL

We have measured the desiccation losses of a large number of aridly stored hog kidneys which had been arterially embalmed with aqueous 2 per cent formaldehyde solutions containing various concentrations of nonvolatile organic solutes. Each kidney was injected through the renal artery with an amount of fluid equal to 37.5 per cent of its fresh weight. After injection the renal artery, vein, and ureter were tied off, and the kidneys were stored at room temperature in large containers over fused calcium chloride. The control kidneys were injected with plain 2 per cent formaldehyde solution, while the fluid used for the others contained in addition, 2.5, 5, and 10 per cent of the following compounds: glycerin, ethylene glycol, diethylene glycol, carbitol, and sorbitol. The kidneys were weighed every day to determine the loss of water from them.

Figs. 1 and 2 show the weight changes in the embalmed material with time in the case of the 10 per cent additions. All the plotted values represent average values on several specimens. Except for minor displacements up or down due to uncontrollable differences in the biological material, the graphs have identical slopes and shapes, and are nearly superimposable. Only slight differences in water loss are found, and we may conclude that very little can be done in reducing the water loss from embalmed bodies unless impracticably large amounts of hygroscopic agents are injected.

REFERENCES

1. Gortner, R. A.: Outlines of Biochemistry, John Wiley & Sons, 1938, p. 287.
2. Cone, R. M., and Davis, L. H.: Determination of the Moisture Content of Tobacco, Indust. & Engin. Chem., Anal. Ed. **9**: 219, 1937.

THE USE OF SS (SHIGELLA-SALMONELLA) AGAR FOR THE ISOLATION OF FLEXNER DYSENTERY BACILLI FROM THE FECES*

HARRY M. ROSE, M.D., AND MAXWELL H. KOLODNY, PH.D.
NEW YORK, N. Y.

SS (SHIGELLA-SALMONELLA) agar is a new culture medium for the isolation of microorganisms of the Shigella, Salmonella, and Eberthella groups from the feces. The medium is of the "selective" type, i.e., it is designed to favor the growth of these pathogens, but to inhibit the coliform bacteria. This selective inhibition of the fecal bacteria enables very large inocula of feces to be used, with a consequently greater chance of cultivating the pathogens when they are present in small numbers. Colonies of pathogens are colorless, whereas any colonies of the nonpathogenic lactose fermenters that develop appear red. The medium is similar in purpose to desoxycholate-citrate agar,[1] the value of which is well-established,[2] but differs considerably from it in composition.†

Mayfield and Gober[3] have furnished the only report yet available on the use of SS agar. These authors examined more than one thousand stool specimens, of which 101 were positive for *E. typhosa*, and 171 were positive for *Sh. dysenteriae*, chiefly of the Flexner group. Each stool specimen was cultured on both desoxycholate-citrate agar and SS agar, and the results on the two media were compared. Of the 272 specimens containing typhoid or dysentery bacilli, 262 were positive on SS agar, and 249 were positive on desoxycholate-citrate agar. Cultures were positive on SS agar alone in 23 specimens, and on desoxycholate-citrate agar alone in 10 specimens. These authors concluded that SS agar was as efficient as desoxycholate-citrate agar for the isolation of dysentery bacilli. They also noted that differentiation between the fecal and pathogenic colonies was more clearly defined on the SS agar, and that the medium was economical.

We have recently had the opportunity to employ SS agar in the bacteriologic study of a small outbreak of Flexner dysentery. Seventeen clinical cases of dysentery were observed, nearly all of which were mild, although several pa-

*From the Departments of Medicine and of Bacteriology, College of Physicians and Surgeons, Columbia University, New York.

Received for publication, December 4, 1941.

†SS agar is a product of the Difco Laboratories, Detroit, Mich. This medium and the others used in this study were purchased on the open market.

It has the following formula:

Beef extract	5.0 Gm.
Proteose peptone	5.0 Gm.
Lactose	10.0 Gm.
Bile salts	8.5 Gm.
Sodium citrate	8.5 Gm.
Sodium thiosulfate	8.5 Gm.
Ferric citrate	1.0 Gm.
Agar	17.0 Gm.
Brilliant green	0.33 mg.
Neutral red	0.025 mg.

Weights of ingredients are per liter. The formula for desoxycholate-citrate agar will be found in reference 1.

tients experienced a severe febrile reaction. Diarrhea persisted in most cases for from three to seven days. The stools contained much mucus, but were rarely bloody. There were no fatalities.

Bacteriologic examinations of the feces from patients and contacts were done in the diagnostic laboratory of the Presbyterian Hospital. This laboratory routinely uses for stool cultures desoxycholate-citrate agar and a nonselective medium, eosin-methylene blue agar. SS agar was introduced shortly after the outbreak began.

A total of 225 stool specimens were examined from patients and contacts. Dysentery bacilli of the Flexner group were isolated from each of the 17 clinical cases and from one of the contacts. Altogether, 70 of the 225 specimens were found to be positive on one or more of the three media. Of the positive specimens, 14 were cultured on eosin-methylene blue agar and desoxycholate-citrate agar. SS agar with either eosin-methylene blue agar or desoxycholate-citrate agar was used for 26 specimens. The remaining 30 specimens were cultured on all three media.

TABLE I

COMPARATIVE EFFICIENCY OF EOSIN-METHYLENE BLUE AGAR, DESOXYCHOLATE-CITRATE AGAR, AND SHIGELLA SALMONELLA AGAR FOR THE ISOLATION OF FLEXNER DYSENTERY BACILLI

70 POSITIVE SPECIMENS COMBINATIONS OF MEDIA USED	RESULTS ON INDIVIDUAL MEDIA					
	EMB AGAR		DC AGAR		SS AGAR	
	NUMBER POSITIVE	NUMBER NEGATIVE	NUMBER POSITIVE	NUMBER NEGATIVE	NUMBER POSITIVE	NUMBER NEGATIVE
EMB and DC, 14 specimens	7	7	13	1	–	–
EMB and SS, 20 specimens	9	11	–	–	20	0
DC and SS, 6 specimens	–	–	5	1	6	0
EMB, DC, and SS, 30 specimens	10	20	25	5	29	1
Total positive and negative on individual media	26	38	43	7	55	1
Per cent positive on individual media	40		86		98	

The results of cultures on the four combinations of media are given in Table I. Eosin-methylene blue agar yielded 26 positives out of 64 cultures in which this medium was used, an incidence of only 40 per cent. The cultures on desoxycholate-citrate agar were positive in 43 of 50 specimens, or 86 per cent. SS agar was positive in 98 per cent of cultures, only one specimen out of 56 being negative on this medium.

The data indicate clearly that a large proportion of the positive specimens would have been considered negative if the nonselective medium alone had been employed. Moreover, most of the isolations on eosin-methylene blue agar were obtained from patients early in the course of their disease, when many dysentery bacilli were being constantly excreted in the stools. After the diarrhea had subsided, however, and the pathogenic bacteria were excreted only intermittently and in small numbers, the value of the selective media became even more apparent. In 7 patients, from two to six weeks after symptoms had disappeared and the persons were apparently healthy, these media revealed 22 positive stools. The same stools were completely negative when cultured on eosin-methylene blue agar.

A comparison of the cultures on the two selective media shows that SS agar was somewhat more efficient than desoxycholate-citrate agar for the isolation of Flexner dysentery bacilli, although the number of specimens examined was relatively small, and no statistical evaluation of the media could be made. However, the SS agar was found to be superior to the desoxycholate-citrate agar in three noteworthy respects: (1) The inhibition of coliform bacteria was more marked; (2) the colonies of dysentery bacilli were more clearly defined; (3) the colonies of dysentery bacilli were usually more numerous.

Our findings, therefore, confirm those of Mayfield and Gober,[3] and indicate that SS agar is a valuable selective medium for the isolation of Flexner dysentery bacilli from the feces.* We feel that the use of SS agar will increase the efficiency of methods for the bacteriologic examination of the feces, a matter of importance to the public health from the standpoint not only of diagnosing enteric disease, but also of recognizing carriers of the intestinal pathogens.

CONCLUSIONS

SS agar is as efficient as desoxycholate-citrate agar for the isolation of Flexner dysentery bacilli.

The medium is superior to desoxycholate-citrate agar as regards inhibition of coliform bacteria, colony differentiation, and growth-promoting qualities for the pathogens.

The combination of SS agar and a suitable nonselective medium furnishes a reliable method for the isolation of the intestinal pathogens. For maximal efficiency two or more plates of the selective medium should be employed.

REFERENCES

1. Leifson, E.: New Culture Media Based on Sodium Desoxycholate for the Isolation of Intestinal Pathogens and the Enumeration of Colon Bacilli in Milk and Water, J. Path. & Bact. **40**: 581, 1935.
2. (a) Paulson, M.: The Clinical Use of Desoxycholate and Desoxycholate-Citrate Agars for the Isolation of Intestinal Pathogens, Am. J. M. Sc. **193**: 688, 1937.
   (b) Hardy, A. V., Watt, James, De Capito, T. M., and Kolodny, M. H.: Studies of the Acute Diarrheal Diseases. I. Differential Culture Media, Pub. Health Rep. **54**: 287, 1939.
   (c) Cooper, M. L., Furcolow, M. L., Mitchell, A. G., and Cullen, G. E.: The Relation of Dysentery to the Acute Diarrhea of Infants and Children, J. Pediat. **15**: 172, 1939.
   (d) Irons, J. V., Bohles, S. W., De Shazo, T., and Hewlett, L. L.: Observations on MacConkey's and Desoxycholate-Citrate Agars for the Isolation of Dysentery Bacilli, J. Lab. & Clin. Med. **25**: 81, 1939.
   (e) Coleman, M. B.: The Differentiation and Identification of Bacillary Incitants of Dysentery, Am. J. Pub. Health **30**: 39, 1940.
   (f) Mayfield, C. R., and Gober, M.: Comparative Efficiency of Endo, Lithium Chloride Endo, Desoxycholate-Citrate, and Bismuth Sulfite Media for the Isolation of Eberthella Typhosa, Am. J. Pub. Health **30**: 69, 1940.
3. Mayfield, C. R., and Gober, Maud: Comparative Efficiency of Plating Media for the Isolation of Shigella Dysenteriae, Am. J. Pub. Health **31**: 363, 1941.

*We have also used SS agar to culture a series of stools from two patients with typhoid fever, one with Sonne dysentery, and one with enteritis caused by *Salmonella panama*. The results indicated that the medium was equally effective for the isolation of these organisms.

A NOTE ON THE "BLACK LINE" IN HEMATOCRIT DETERMINATIONS*

J. Percy Baumberger,† D.Sc., Stanford University, Calif.

IT MAY be a common observation that a narrow black band appears in hematocrit determinations at the top of the erythrocyte column, though the textbooks of hematology[1-6] and the literature[7-9] here listed make no reference to it. Neither Hedin in his pioneer work on the hematocrit nor Koeppe, who first described the development of translucence of erythrocytes when blood is centrifuged in hematocrit tubes at high speed, makes mention of the black line. It is, therefore, considered worth while to mention this phenomenon and to describe some simple experiments which establish its nature.

Oxalated human blood drawn directly from the cubital vein is introduced into Wintrobe hematocrit tubes capped with rubber, and centrifuged at about 2,500 r.p.m. for ten minutes. The tubes then show a lower column of erythrocytes and an upper column of plasma with about one division (1 per cent) of leucocytes above the erythrocytes. Immediately below the leucocytes a dark line is present, varying in intensity and width, but usually equal to about 0.5 per cent by volume. When hemolysis is great, black lines are seen both above and below the leucocytes.

The plasma and leucocytes may be pipetted off without disturbing the black layer, but it disappears on standing exposed to air, becoming red. If saline solution is now added to the 100 per cent mark, and the erythrocytes are resuspended and then centrifuged down, no dark line appears, obviously because there are no leucocytes to consume the oxygen. However, if a few yeast cells, *S. cerevisiae,* are now added to the top of the saline, in the above experiment, and are then centrifuged down, so that they rest on the top of the erythrocyte column, the dark band reappears as a result of oxygen consumption by the yeast cells. If blood is chilled and, while ice cold, is introduced into the hematocrit, on centrifugation, no black line appears at first but develops after a few minutes' standing at room temperature, when it increases in width gradually until a maximum width is reached. If the temperature is now increased to 37° C., the black line again increases in width with time, finally reaching a new maximum width of not more than 1 per cent. The width of the line is a function of the rate of oxygen consumption by the leucocytes as influenced by temperature.

Evidently the black line is a layer of erythrocytes in which the oxyhemoglobin has been reduced to hemoglobin by the metabolic activity of the leucocytes.

*From the Research Department of the Barnard Free Skin and Cancer Hospital, St. Louis, Mo.

†Present address: Physiology Department, Stanford University, Calif.

The black line is thus reversible and can be produced by any agency using up oxygen locally at a rate greater than the diffusion rate of oxygen in the plasma or from distant erythrocytes. Perhaps the rate of increase in width of the line may be a useful indicator. For instance, if the width of the line is unequal in two samples of blood, while the leucocytic count is equal, one would expect a qualitative difference in the leucocytes.

I wish to acknowledge the helpful advice of Dr. Carl Moore, Washington University Medical School, and the kindness of Dr. W. C. Herold, St. Louis, in furnishing blood samples.

REFERENCES

1. Beck, R. C.: Laboratory Manual of Hematologic Technic, Philadelphia, 1938, W. B. Saunders Co.
2. Haden, R. L.: Principles of Hematology, ed. 2, Philadelphia, 1940, Lea & Febiger.
3. Kolmer, J. A., and Boerner, F.: Approved Laboratory Technique, ed. 2, New York, 1938, D. Appleton-Century Co.
4. Magner, W.: A Textbook of Hematology, Philadelphia, 1938, P. Blakiston's Son & Co.
5. Osgood, E. E.: Textbook of Laboratory Diagnosis, ed. 2, Philadelphia, 1935, P. Blakiston's Son & Co.
6. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis by Laboratory Method, ed. 9, Philadelphia, 1939, W. B. Saunders Co.
7. Wintrobe, M. M., and Landsberg, J. W.: A Standard Technique for the Blood Sedimentation Test, Am. J. M. Sc. **189**: 102, 1935.
8. Hedin, S. G.: Der Hematocrit, ein neuer Apparat zur Untersuchung des Blutes, Skandinav. Arch. f. Physiol. **2**: 134, 1890.
9. Koeppe, H.: Über das Lackfarbenwerden der roten Blutscheiben, Pflüger's Arch. f. d. ges. Physiol. **107**: 183, 1905.

CHEMICAL

A MICROBIOLOGICAL ASSAY METHOD FOR SIX B VITAMINS USING LACTOBACILLUS CASEI AND A MEDIUM OF ESSENTIALLY KNOWN COMPOSITION*

MAURICE LANDY, PH.D., AND DOROTHY M. DICKEN, M.A.
CHAGRIN FALLS, OHIO

IT IS generally agreed that there is a great need in current vitamin research for rapid methods for the quantitative determination of vitamins. This is particularly true in the case of the vitamins of the B complex.

Since many B complex vitamins required by animals are essential in bacterial metabolism, microbiological assays, i.e., vitamin assays employing microorganisms as test agents, have rapidly gained approval. As our knowledge of bacterial nutrition has accumulated, these procedures have increased in number and have been revised and amplified. Microbiological assays are not only extremely sensitive to minute amounts of test material, but are accurate as well, and have the additional advantage over animal assays in being rapid and inexpensive. Furthermore, in the case of folic acid, microbiological technique is the only known method of assay.

Microbiological procedures in current use include assays for riboflavin *(Lactobacillus casei)*,[1] pantothenic acid *(L. casei)*,[2] pyridoxine *(Saccharomyces cerevisiae)*,[3] nicotinic acid *(L. arabinosus)*,[4] folic acid *(Streptococcus lactis)*,[5] and biotin *(S. cerevisiae, Clostridium butylicum)*.[6, 7] During the preparation of this manuscript a paper by Shull, Hutchings, and Peterson[8] describing a microbiological assay for biotin utilizing *L. casei* appeared. From the foregoing examples it is obvious that microbiological assays of the various B vitamins have necessitated use of a variety of organisms, culture media, and test conditions. Although the majority of these assays has been satisfactory, it would obviously be advantageous to have a single acidimetric method for members of the B complex, using only one test organism and a simplified basal medium of known composition. The organism presenting the greatest possibilities as an assay agent under these conditions is *L. casei*† because (1) its nutritional requirements have been extensively investigated and have been found to include a majority of the B complex vitamins; (2) its acid production, which is easily and accurately determined, is a function of its growth; and (3) many laboratories are already familiar with its use in riboflavin and pantothenic acid assays.

At the time this study was begun, the status of the nutrition of *L. casei* was as follows: It had been demonstrated that this organism required ribo-

*From the Research Laboratories, S. M. A. Corporation, Chagrin Falls, Ohio.
Received for publication, February 27, 1942.
†American type culture collection No. 7469.[9]

flavin,[1] pantothenic acid,[10] nicotinic acid,[10] and pyridoxine.[11] The essential growth factors of unknown composition, other than those possibly present in hydrolyzed casein, were supplied by yeast extract and could be separated by adsorption on norit A into "filtrate" and "eluate" fractions.[11] Evidence had been presented indicating that the activity of the eluate fraction was due to "folic acid."[5, 12] However, the composition of the filtrate fraction was still unknown.

We, therefore, undertook (1) replacement of the yeast filtrate fraction with known substances which would permit maximum growth of *L. casei* in a medium of essentially known composition, and (2) thus, having determined the nutritional requirements of *L. casei*, to use this information in establishing assay procedures for the various vitamins this organism requires.

EXPERIMENTAL

At the time our studies on the nutrition of *L. casei* were begun, an investigation was in progress in this laboratory on the use of avidin in determining the biotin requirement of microorganisms.[13] A correlation had been established between growth inhibition by avidin and the biotin requirement of an organism. In general, those organisms which have been found to require biotin supplied in the culture medium were inhibited by avidin, while those organisms which were found to synthesize biotin[14] were not affected. The growth and acid production of *L. casei* were completely inhibited by avidin, and this inhibition could be readily reversed by the subsequent addition of crystalline biotin. This was taken as evidence that *L. casei* requires biotin as a growth essential.

Employing the basal medium of Snell and Peterson[11] containing pyridoxine and filtrate fraction of yeast extract as prepared by them, and with their norit eluate fraction replaced by "folic acid" in excess, we found that biotin would replace the filtrate fraction, yielding equivalent growth. Since we have not always been able to obtain maximum growth with the filtrate fraction, it was suspected that this constituent might contain insufficient biotin to support maximum growth. Biotin assay[6] of the filtrate fraction revealed that when prepared and used according to Snell and Peterson, a culture would be supplied with 0.0001γ of biotin. This amount was subsequently found insufficient for maximum growth of *L. casei*.* Peterson has recently stated, "the filtrate fraction can be replaced by biotin, thus leaving the eluate fraction as unknown."[12] However, no evidence was offered to support this statement.

In an effort to obtain maximum growth consistently the effect of a variety of growth accessory substances was investigated. As a result of the findings of Snell and Mitchell[15] that purine and pyrimidine bases are growth substances for lactic acid bacteria, adenine, guanine, xanthine, and uracil were included in the medium. These bases, together with biotin and folic acid concentrate, consistently supported maximum growth. We later added asparagine, since Feeney and Strong[16] had found it to be a growth accelerator, particularly

*From our tests with Snell and Peterson's filtrate fraction, and as a result of biotin assay of this material, it would appear likely that under their conditions of test, the filtrate fraction supplied only a portion of the total biotin necessary for maximum growth. The remainder of the biotin necessary for maximum growth was probably supplied by their norit eluate concentrates, since their method of preparation might also concentrate biotin.

during the first twenty-four hours of incubation. The vitamins and growth accessories, as given in Table I, completely replace yeast extract, which is considered[11] the best source of growth essentials for supporting maximum growth and acid production of *L. casei*.

TABLE I

COMPLETE MEDIUM FOR LACTOBACILLUS CASEI

Casein hydrolysate	0.5 Gm. (dry weight)
Sodium acetate ($NaC_2H_3O_2 . 3 H_2O$)	0.6 Gm.
Glucose	1.0 Gm.
Asparagine	25 mg.
Tryptophane	10 mg.
Cystine	10 mg.
Salt solution A	0.5 c.c.
Salt solution B	0.5 c.c.
Guanine hydrochloride	500 μg
Adenine sulfate	500 μg
Xanthine	500 μg
Uracil	500 μg
Thiamine hydrochloride	10 μg
Biotin (free acid)	0.5 μg
Folic acid concentrate (50 per cent folic acid)*	1.0 μg
Riboflavin	20 μg
Calcium pantothenate	20 μg
Nicotinic acid	20 μg
Pyridoxine hydrochloride	40 μg
Distilled water to	100 c.c.

pH adjusted to 6.8

Salt solution A		Salt solution B		
K_2HPO_4	5 Gm.	$MgSO_4 . 7 H_2O$	10	Gm.
KH_2PO_4	5 Gm.	NaCl	0.5	Gm.
Water	50 c.c.	$FeSO_4 . 7 H_2O$	0.5	Gm.
		$MnSO_4 . 2 H_2O$	0.337	Gm.
		Water	250	c.c.

*Kindly supplied by Dr. R. J. Williams, Department of Chemistry, University of Texas, Austin, Texas.

Medium.—The composition of our complete basal medium is given in Table I. It is essential that all constituents be of the highest purity. Best results are obtained if the medium is prepared just before use. Stock solutions of tryptophane, cystine hydrochloride, salt solutions A and B, and purine bases may be kept on hand, using toluene as a preservative where necessary. Since the purine and pyrimidine bases are not readily soluble in water, it has been found convenient to dissolve them in the casein hydrolysate. After the addition of riboflavin to the medium, exposure to light should be avoided.

It is necessary that great care be taken in the preparation of the casein hydrolysate, since this is the only constituent whose composition is not chemically defined; it is, therefore, the only source of variation. Purified or "vitamin-free casein"* should be reprecipitated several times in order to remove traces of interfering vitamins, particularly nicotinic acid and pyridoxine. Reprecipitation is followed by the usual acid hydrolysis, and the resulting hydrolysate is treated with activated carbon, primarily for the removal of any biotin present. Culture medium containing casein hydrolysate prepared in this fashion supports maximum growth of the test organism and yields satisfactory blanks for all the six vitamin assays.

*Most caseins of this type are "vitamin free" to the extent of being satisfactory for animal assays, but they contain small quantities of vitamins sufficient to interfere with microbiological assays.

Reprecipitation of Casein.—To one liter of distilled water at 50° C. 100 Gm. casein are added gradually with stirring. This mixture is stirred for thirty minutes, 3 Gm. of sodium bicarbonate (dissolved in a convenient volume of water) are added, and the stirring is continued until the casein is dissolved. It may be necessary to add more water. When the solution is complete, the pH is adjusted to 4.6 with 10 per cent hydrochloric acid. The casein precipitate is allowed to settle and is removed by filtration. The reprecipitation procedure is repeated several times, depending upon the purity of the original casein. As a rule, several reprecipitations are necessary before satisfactory blanks can be obtained in culture medium prepared with the resulting hydrolysate.

Preparation of Casein Hydrolysate.—One hundred grams (dry weight) of the reprecipitated casein are hydrolyzed with 500 c.c. of 25 per cent sulfuric acid for ten hours at 15 pounds pressure. The sulfuric acid is neutralized with barium hydroxide and the barium sulfate is removed by filtration.

*Treatment of Casein Hydrolysate With Activated Carbon.**—Casein hydrolysate solution in a concentration of 10 per cent (total solids) is acidified to pH 3 and treated with Nuchar (1 Gm. of Nuchar to 10 Gm. of casein). The mixture is stirred for one hour and filtered through Filter-cel. The resulting hydrolysate may be preserved under toluene or sterilized by autoclaving.

Assay Procedure.—When we succeeded in obtaining maximum growth of *L. casei* in a medium of essentially known composition, we turned to the problem of establishing assay procedures. It was found that, for any one essential vitamin, growth and acid production were consistently proportional, within a limited range, to the amount of vitamin supplied. This quantitative response formed the basis for the following assay method:

For the assay of any one of the six vitamins, basal medium of twice the concentration given in Table I is prepared with the omission of the vitamin under test. Dilutions of the sample to be assayed are placed in culture tubes with sufficient distilled water to make a total volume of 5 c.c. in each tube. Five cubic centimeters of medium (twofold concentration) are added, giving a final total volume of 10 c.c. per tube. Similarly, dilutions of the vitamin used as a standard are prepared. The tubes are plugged with cotton and sterilized at 15 pounds pressure for fifteen minutes. After cooling, the tubes are inoculated with a suspension of *L. casei* and are incubated at 37.5° C. for seventy-two hours. Following incubation, the amount of growth is determined by titration of the acid produced, using 0.1 N sodium hydroxide. Titration may be colorimetric with bromthymol blue as the indicator, or electrometric, using either glass or quinhydrone electrode. Values obtained from the dilutions of the vitamin standard are used to construct a standard curve from which the vitamin content of any dilution of sample may be calculated. Typical standard curves for all six vitamins required by *L. casei* are presented in Fig. 1.

Preparation of Inoculum.—Stock cultures of *L. casei* are carried in yeast dextrose agar stabs and are transferred as recommended by Snell and Strong.[1] A twenty-four-hour culture of *L. casei* in 10 c.c. of yeast dextrose broth is centrifuged, and the bacteria are washed in 10 c.c. of 0.85 per cent sodium

*Nuchar XXX, Industrial Chemical Sales Co., Inc., 230 Park Ave., New York, N. Y.

chloride solution. The washed sediment is resuspended in 4 c.c. of saline, and 1 c.c. of the resulting suspension is added to 85 c.c. of saline. One-tenth cubic centimeter of this dilute suspension per tube serves as the inoculum.

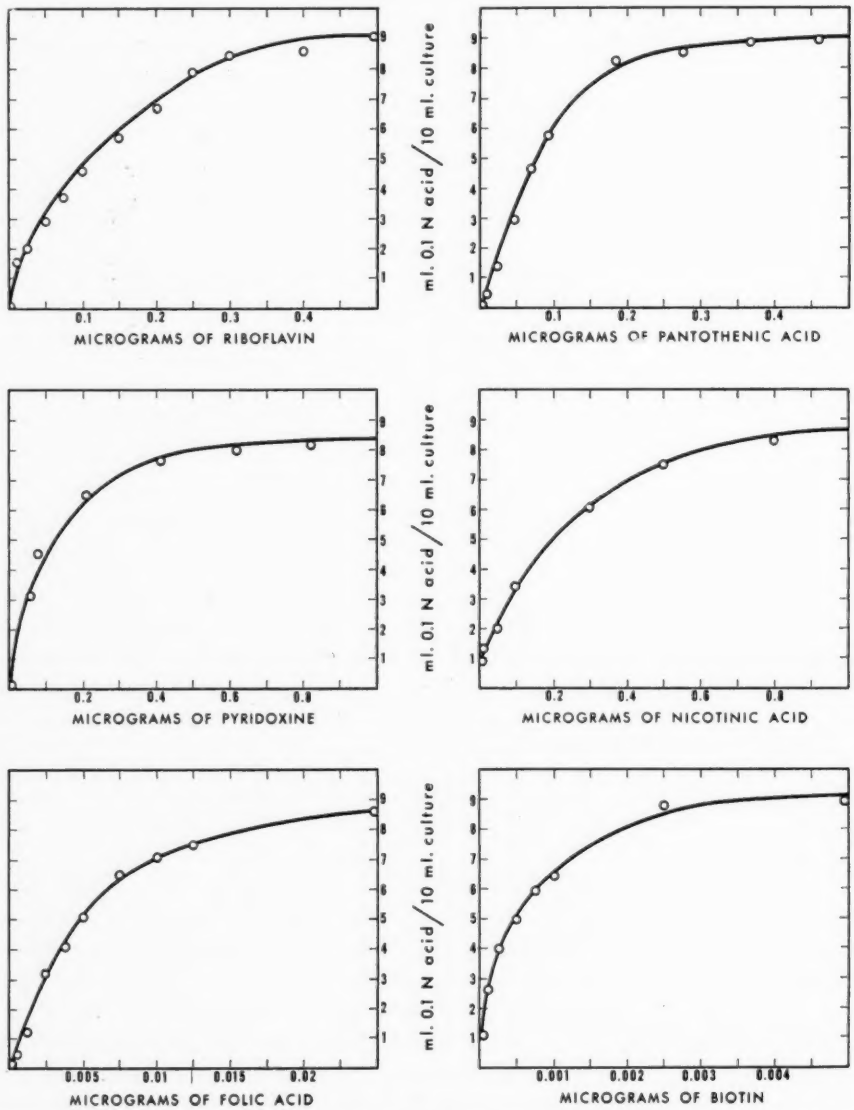

Fig. 1.—Standard assay curves for six B vitamins using *Lactobacillus casei* and a medium of essentially known composition.

DISCUSSION

From the data presented, it is clear that excellent growth of *L. casei* is obtained when riboflavin, pantothenic acid, nicotinic acid, folic acid, pyridoxine, and biotin are supplied in a suitable basal medium. Omission of any one of these essential vitamins will result in absence of growth and acid production. In the case of pantothenic acid, riboflavin, folic acid, and pyridoxine perfect blanks (no growth or acid production) were obtained. In the nicotinic acid

and biotin blanks a small amount of growth occurred, equivalent to approximately 1.0 c.c. of 0.10 N acid. Blanks of this magnitude are not prohibitive and do not interfere with accurate assay determinations. These blanks are due to traces of nicotinic acid and biotin which may ultimately be removed from the constituents of the medium.

For all six vitamins growth and acid production are, within a limited range, proportional to the concentration of the vitamin under test. The extreme upper and lower portions of the standard curve do not give reliable results, and only the portion of the curve which is linear, or approximately so, should be used for the calculation of assay values. When solutions or extracts of samples are run at several dilutions that fall within the accurate portion of the standard curve, values obtained at these different levels check closely.

The amount of each essential vitamin necessary for detectable growth of *L. casei* differs from one vitamin to another, but is minute in every case. This response to such small quantities provides sensitive tests for these vitamins. Because of this sensitivity, a sample to be assayed may be considerably diluted, thus eliminating or reducing the effect of nonspecific growth-stimulatory substances.

Preparation of the sample is an important part of the test, and one which, in the past, has not received the attention it merits. Liquid samples or water-soluble materials may be assayed without further treatment. Where the sample is particulate, autoclaving in large volumes of water at acid pH will, as a rule, provide quantitative extracts of most of the B complex vitamins. However, where the vitamin exists in combined form, as in the case of biotin, it is necessary to hydrolyze the sample in order to release the vitamin for utilization by *L. casei*. The methods for preparation of samples and the importance of such procedures, especially for biotin assay, have recently been reviewed.[7, 17-19]

The vitamin content of various naturally occurring materials and concentrates was determined both by our assay procedure and by established microbiological methods. Such parallel comparison assays of yeast and rice bran concentrate for riboflavin by the Snell and Strong method and for pantothenic acid by the method of Pennington, Snell, and Williams were in excellent agreement, while results of our biotin assay of a biotin concentrate compared favorably with values obtained by the yeast growth method of Snell, Eakin, and Williams. Although comparison with other methods has not been made for nicotinic acid, folic acid, and pyridoxine, it is probable that satisfactory results would be obtained, since the response of *L. casei* to these vitamins is similar in sensitivity, magnitude of blank, and type of standard curve to that obtained with riboflavin, pantothenic acid, and biotin.

SUMMARY

1. Maximum growth and acid production of *L. casei* have been obtained in a medium in which all constituents, except casein hydrolysate, are chemically defined.

2. Using this medium of essentially known composition with *L. casei* as the assay agent, it has been possible to establish a general assay procedure

for the vitamins essential for growth and acid production by this organism, *viz.*, pantothenic acid, riboflavin, nicotinic acid, pyridoxine, folic acid, and biotin.

REFERENCES

1. Snell, E. E., and Strong, F. M.: A Microbiological Assay for Riboflavin, Indust. & Engin. Chem. (Anal. Ed.) **11**: 346, 1939.
2. Pennington, D., Snell, E. E., and Williams, R. J.: An Assay Method for Pantothenic Acid, J. Biol. Chem. **135**: 213, 1940.
3. Williams, R. J., Eakin, R. E., and McMahan, J. R.: Assay Method for Pyridoxin, University Texas Publication No. 4137, 24, 1941.
4. Snell, E. E., and Wright, L. D.: A Microbiological Method for the Determination of Nicotinic Acid, J. Biol. Chem. **139**: 675, 1941.
5. Mitchell, H. K., Snell, E. E., and Williams, R. J.: The Concentration of "Folic Acid," J. Am. Chem. Soc. **63**: 2284, 1941.
6. Snell, E. E., Eakin, R. E., and Williams, R. J.: A Quantitative Test for Biotin and Observations Regarding Its Occurrence and Properties, J. Am. Chem. Soc. **62**: 175, 1940.
7. Lampen, J. O., Bahler, G. P., and Peterson, W. H.: The Occurrence of Free and Bound Biotin, J. Nutrition **23**: 11, 1942.
8. Shull, G. M., Hutchings, B. L., and Peterson, W. H.: A Microbiological Assay for Biotin, J. Biol. Chem. **142**: 913, 1942.
9. Tittsler, R. P., Rogosa, M., and Whittier, E. O.: The Nomenclature and Characteristics of the Lactobacillus Employed for the Microbiological Assay of Riboflavin, J. Bact. **43**: 56, 1942.
10. Snell, E. E., Strong, F. M., and Peterson, W. H.: Growth Factors for Bacteria. VIII. Pantothenic and Nicotinic Acids as Essential Growth Factors for Lactic and Propionic Acid Bacteria, J. Bact. **38**: 293, 1939.
11. Snell, E. E., and Peterson, W. H.: Growth Factors for Bacteria. X. Additional Factors Required by Certain Lactic Acid Bacteria, J. Bact. **39**: 273, 1940.
12. Hutchings, B. R., Bohonos, N., and Peterson, W. H.: Growth Factors for Bacteria. XIII. Purification and Properties of an Eluate Factor Required by Certain Lactic Acid Bacteria, J. Biol. Chem. **141**: 521, 1941.
13. Landy, M., Dicken, D. M., Bicking, M. M., and Mitchell, W. R.: The Use of Avidin in Studies on the Biotin Requirement of Microorganisms, J. Bact. **43**: 5, 1942.
14. Landy, M., and Dicken, D. M.: Biotin Synthesis by Microorganisms, Proc. Soc. Exper. Biol. & Med. **46**: 449, 1941.
15. Snell, E. E., and Mitchell, H. K.: Purine and Pyrimidine Bases as Growth Substances for Lactic Acid Bacteria, Proc. Nat. Acad. Sc. **27**: 1, 1941.
16. Feeney, R. E., and Strong, F. M.: Identification of Growth-Stimulating Substances for *Lactobacillus casei*, J. Biol. Chem. **140**: xxxviii, 1941.
17. Pennington, D., Snell, E. E., Mitchell, H. K., McMahan, J. R., and Williams, R. J.: Assay Method for Pantothenic Acid, University Texas Publication No. 4137, 14, 1941.
18. Thompson, R. C., Eakin, R. E., and Williams, R. J.: The Extraction of Biotin From Tissues, Science **94**: 589, 1941.
19. Gyorgy, P., Kuhn, R., and Lederer, E.: Attempts to Isolate the Factor (Vitamin H) Curative of Egg White Injury, J. Biol. Chem. **131**: 745, 1939.

ON THE DETERMINATION OF URINARY CHOLESTEROL*

MAURICE BRUGER, M.D., AND SYLVIA B. EHRLICH, A.B.
NEW YORK, N. Y.

IN 1933, Mirsky[1] described a colorimetric procedure for the estimation of urinary cholesterol based upon the observation of de Toni,[2] who found that the tungstic acid precipitate of blood contained all the cholesterol. The method proposed by Mirsky utilized the addition of a 50 per cent solution of sodium tungstate to urine, and the subsequent slow addition of concentrated sulfuric acid to precipitate the tungstic acid. When observations on the excretion of cholesterol in the urine in patients with cancer were begun in this laboratory,[3] it was found that this procedure, at times, failed to recover satisfactory amounts of serum cholesterol added to the urine. It was assumed that urines, particularly from persons without renal disease, contained an insufficient amount of protein to carry down all the cholesterol. The addition of egg albumin to the urine was found to be a necessary step in order to obtain satisfactory recoveries of added cholesterol.

Preparation of Egg Albumin Solution.—The separation of the egg albumin from the yolk must be done carefully, since it is known that egg yolk contains appreciable quantities of cholesterol. The egg white was diluted with 9 volumes of distilled water and the solution was filtered through fluted filter paper. The cholesterol content of each batch of diluted egg albumin was determined; small amounts of cholesterol were invariably present, but the quantities in different batches were found to be remarkably constant (0.15 mg. in 100 c.c. of diluted egg albumin solution). Corrections for the cholesterol content of the added egg white were made in final calculations.

METHOD

One hundred cubic centimeters of urine are placed in a 150 c.c. beaker or other suitable container. The urine is acidified with 0.5 c.c. of concentrated sulfuric acid, and 10 c.c. of the diluted egg albumin solution are added. After mixing, the albumin is precipitated by the addition of 5 c.c. of 10 per cent solution of sodium tungstate. The contents are again well mixed, and the precipitate is allowed to settle out for five to ten minutes. The solution is now filtered through a fluted filter paper (previously rendered fat free by washing in alcohol and ether). The protein precipitate is washed repeatedly with hot distilled water; the extent of the washing varies with the quantity of urinary pigment in the protein coagulum (50 c.c. to 100 c.c. of water usually suffice).

*From the Medical Research Laboratory, Department of Medicine, New York Post-Graduate Medical School and Hospital, Columbia University, New York.
Received for publication, January 28, 1942.

The precipitate and filter paper are transferred to a 150 c.c. beaker, and 30 c.c. of a 3:1 alcohol-ether mixture are added. This is allowed to stand for fifteen to thirty minutes or, if more convenient, overnight. The beaker is then placed on an electric hot plate. The contents are brought to a boil and are then filtered through fat-free filter paper into another beaker of the same capacity.

TABLE I

RECOVERY OF ADDED SERUM CHOLESTEROL

NO.	URINE		DILUTED EGG ALBUMIN ADDED		SERUM CHOLESTEROL ADDED (MG.)	URINARY CHOLESTEROL		GAIN OR LOSS (%)
	VOL. (C.C.)	CHOLESTEROL (MG.)	VOL. (C.C.)	CHOLESTEROL (MG.)		CALCULATED (MG.)	FOUND (MG.)	
I	100	0.223	10	0.015	0.133	0.371	0.397	+7
	100	0.223	10	0.015	0.266	0.504	0.458	−9
	100	0.223	10	0.015	0.398	0.636	0.620	−2
	100	0.223	10	0.015	0.531	0.769	0.781	+1
II	100	0.128	10	0.015	0.174	0.317	0.298	−6
III	100	0.171	15	0.023	0.133	0.327	0.324	0
	100	0.171	15	0.023	0.266	0.460	0.463	0
	100	0.171	15	0.023	0.398	0.592	0.551	−6
	100	0.171	15	0.023	0.531	0.725	0.688	−5
IV	100	0.141	10	0.015	0.664	0.820	0.806	−2
	100	0.141	10	0.015	0.797	0.953	0.993	+4
V	100	0.114	10	0.015	0.930	1.059	1.063	0
	100	0.114	10	0.015	1.063	1.192	1.230	+3
	100	0.114	10	0.015	1.195	1.324	1.351	+2

TABLE II

DUPLICATE DETERMINATIONS

VOL. OF URINE USED (C.C.)	TOTAL CHOLESTEROL (MG.)		TOTAL CHOLESTEROL (MG./100 C.C.)		MEAN DEVIATION (%)
	A	B	A	B	
2 × 300	0.670	0.714	0.223	0.238	5.3
2 × 300	0.682	0.688	0.227	0.229	0.4
2 × 300	0.701	0.707	0.234	0.236	0.4
2 × 300	3.947	3.750	1.316	1.250	2.6
2 × 300	0.444	0.460	0.148	0.153	1.6
2 × 300	0.559	0.547	0.186	0.182	1.1
2 × 300	0.556	0.559	0.185	0.186	0.3

Two additional extractions with alcohol-ether are made, and the filtrates are combined. The alcohol-ether extract is placed in an incubator (37° C.) and evaporated to dryness. To the dry residue in the beaker 30 c.c. of petroleum ether (B. P. 30° to 50° C.) are added. The beaker is placed on a hot plate and the petroleum ether is evaporated to approximately 10 c.c. volume. This is filtered through fat-free cotton or filter paper into another beaker; two additional petroleum ether extractions are carried out, and extracts are combined. This extract is placed in an incubator (37° C.) and is evaporated to dryness. The residue is extracted with 5 c.c. of chloroform, the chloroform extract is transferred to a graduated cylinder of 10 c.c. capacity; 2 c.c. of acetic anhydride and 0.1 c.c. of concentrated sulfuric acid are added. A standard solution containing 0.5 mg. of cholesterol in 5 c.c. of chloroform is prepared simultaneously for the development of the Liebermann-Burchard color reaction.[4] A red filter (Wratten 71-A) is used to facilitate color matching.

Calculation.— $\frac{R_s}{R_u} \times 0.5 \times \frac{100}{X}$ = mg. cholesterol in 100 c.c. of urine

Where R_s = reading of standard
R_u = reading of unknown
X = volume of urine extracted

For the determination of cholesterol in normal urine approximately 300 c.c. of urine must be extracted if the twenty-four-hour-urine volume does not exceed 1,500 c.c. With dilute urines, larger amounts must be extracted. This is done, preferably, by working with 100 c.c. quantities and combining the petroleum ether extracts.

RESULTS

Tables I and II are self-explanatory. The satisfactory recovery of added cholesterol to urine and the close correlation of duplicate determinations are shown in the protocols.

SUMMARY

A satisfactory colorimetric procedure for the determination of urinary cholesterol employing the addition of egg albumin to bind the cholesterol is described.

REFERENCES

1. Mirsky, I. A.: A Method for the Colorimetric Determination of the Urinary Cholesterol, J. Lab. & Clin. Med. **18**: 1068, 1933.
2. de Toni, G. M.: The Colorimetric Estimation of Cholesterol and Lecithin in Blood in Connection With Folin-Wu's System of Blood Analysis, J. Biol. Chem. **70**: 207, 1926.
3. Bruger, M., and Ehrlich, S. B.: Unpublished results.
4. Mirsky, I. A., and Bruger, M.: A Note on the Liebermann-Burchard Color Reaction for Cholesterol, J. Lab. & Clin. Med. **18**: 304, 1932.

AN IMPROVED PYKNOMETER FOR BLOOD SERUM*

R. A. Mortensen, Ph.D., Loma Linda, Calif.

FOR the direct determination of the specific gravity of blood serum, as well as for the standardization and checking of the various indirect methods, a specially constructed specific gravity bottle, introduced by Moore and Van Slyke,[1] has been extensively used. However, precise adjustment of the volume of the liquid is generally troublesome. The uncertainty of seating of the ground stopper and the tendency of serum to seep up by capillary attraction between the stopper and the neck cause significant errors unless time-consuming measures are taken for their prevention. These difficulties are not encountered in a pyknometer of the pipette type, such as the one employed by Brown and Clark,[2] but it is always necessary to adjust the meniscus in the capillary exactly to a calibration mark. The pyknometer here described provides a more rapid and precise measurement by utilizing the principle of automatic volume adjustment employed in the well-known Trenner blood-diluting pipette.

This pyknometer, which is not difficult to construct, consists of a thin-walled bulb *D* sealed to bent capillary tubes *C* and *E*, as shown in Fig. 1. The internal diameter of *E* is about 0.5 mm., and that of *C* is somewhat greater, but the tip *A* is drawn down to approximately the same size as *E*. The end of the capillary at *H* is a ground and polished surface at right angles to the longitudinal axis, and represents the point to which the liquid is brought in filling the device. A small bulb *F* serves to prevent the liquid from entering the capillary *G* too rapidly after filling the main bulb.

Sizes from 1 to 2 ml. have been found to be satisfactory. If many samples are to be measured, a considerable saving in time may be effected by constructing the pyknometer to contain an integral number of grams of water at 20° C. This is accomplished conveniently by expanding or shrinking the bulb before bending the capillary stems. Subsequent bending of the tubes produces a slight decrease in volume, permitting final adjustment to be made by enlarging the capillary at *B* and grinding the tip.

In using the pyknometer, a rubber suction tube is attached to the end *I*, and the serum contained in the short test tube *T* is drawn up to the small bulb *B*. The test tube is then tilted at an angle while the end *E* is lowered, permitting the liquid to flow to *H* by gravity and capillary action. The pyknometer is next removed from the test tube and quickly wiped with a damp cloth, while *A* is maintained at a slightly higher level than *H* in order to lessen the chance of drawing the liquid away from the latter.

Measurements are made at room temperature and the weight at 20° C. is estimated by applying a suitable correction. The number of milligrams to be

*From the Department of Biochemistry, School of Medicine, College of Medical Evangelists, Loma Linda.

Received for publication, February 20, 1942.

added to, or subtracted from, the weight at room temperature to give the weight at 20° C. may be obtained from a chart prepared by Moore and Van Slyke.[1, 3] In order to avoid warming the liquid, the pyknometer is filled as quickly as possible, care being taken to handle it only by the stems at points well removed from the bulb. Loss of liquid due to expansion during weighing may be prevented by forcing a small drop out of the capillary at *H*. With these precautions satisfactory results can be obtained without the use of a thermostat. Evaporation of the serum will ordinarily cause no significant decrease in weight. In one test the loss was less than 0.1 mg. when the filled pyknometer was left in the balance case for one-half hour.

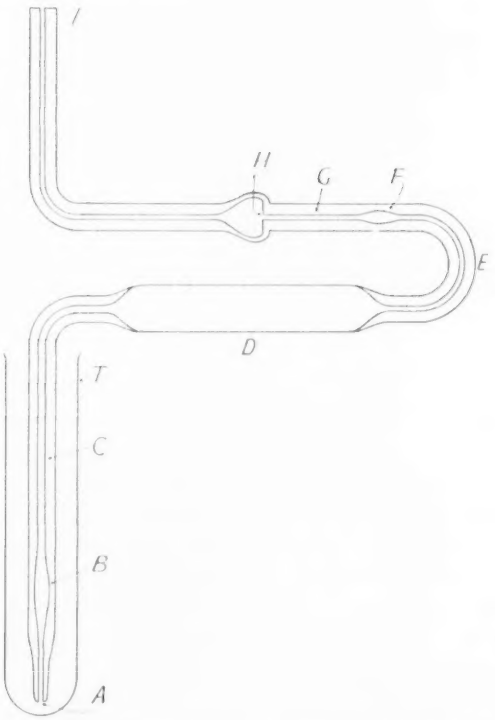

Fig. 1.—Pipette pyknometer with automatic volume adjustment.

The pyknometer is capable of yielding results which are accurate within one or two units in the fourth decimal place, with a minimum expenditure of time and effort. In a long series of measurements on the same sample, using the 1 ml. size which was cleaned, dried, and refilled for each determination, the maximum deviation from the mean specific gravity was less than 2×10^{-4}.

Although designed for use with blood serum, the device obviously may be employed for determining the specific gravity of other aqueous solutions.

REFERENCES

1. Moore, N. S., and Van Slyke, D. D.: The Relationship Between Plasma Specific Gravity, Plasma Protein Content and Edema in Nephritis, J. Clin. Investigation **8**: 337, 1930.
2. Brown, H. R., Jr., and Clark, W. F.: Plasma Specific Gravity and Control of Fluid Administration in Artificial Fever, Proc. Soc. Exper. Biol. & Med. **40**: 490, 1939.
3. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Vol. II. Methods, Baltimore, 1932, Williams & Wilkins Company.

MEDICAL ILLUSTRATION

WAX-RESIN COMPOSITIONS FOR MOULAGE-MAKING

CARL DAME CLARKE, PH.D., BALTIMORE, MD.

IN THE April, 1942, issue of this JOURNAL[1] I, together with a co-worker, described a basic agar composition for making molds or negatives of living and dead tissue to be used in moulage work. Since a cast or a positive is made in such a mold, I wish to confine the present article to the basic materials used in making the positive. There are numerous materials that can be employed for this purpose, such as wax-resin mixtures, celluloid, rubber, and a synthetic fleshlike gelatin mixture. Rubber has been discussed in a previous article.[2] Celluloid, as well as wax-resin mixtures, has been discussed to some extent in my book *Molding and Casting*.[3] Because of the shortage of rubber, experiments are in progress at the present time to develop a suitable substitute for making both positive and negative moulages. This work will be published at a later date.

The average novice has had greater success in working with waxes and resins for the positive; therefore, these materials will be considered here in some detail. Furthermore, the wax-resin mixtures lend themselves more readily to corrections. They also can be used over again in the event of a failure. When the moulage worker has become thoroughly familiar with rubber, celluloid, and gelatin mixtures, he will find that they are as simple to compound and use as the wax-resin mixtures. All these materials, when properly used, possess the translucency of flesh, which is the first essential in choosing any positive composition. In contrast to these materials, plaster of Paris and papier-mâché, which are among the most common positive materials, lack this translucency, and, for this reason, cannot be used with the same degree of success in making facsimile reproductions of protoplasmic objects.

Probably the greatest drawback to the use of wax-resin mixtures is the fact that the resulting cast is broken easily. This disadvantage is overcome when the cast is made of celluloid, rubber, or gelatin mixtures. However, the wax-resin cast is important because it is often used as an intermediate step in making casts of other materials. For example, in a rubber facial prosthesis a wax cast generally is made first and fitted to the patient before the rubber one is made. Necessary changes to obtain perfect fitting are easier to effect in wax than in rubber. Accordingly, a basic wax-resin formula will be considered in detail.

The wax-resin composition used for the positive impression should possess the following qualities:

1. It should be possible to brush or "paint" the positive composition into a plaster or agar mold without having the cast crack or lift from the surface of the mold.

2. The melting point should be so high that it will not be affected by natural heat in the hottest weather. A cast, no matter how thin, should never droop as a candle often does under normal summer temperatures.

3. It should not crack or shrink to any great extent when subjected to the normal cold of winter.

4. It should not crack under sudden changes of temperature, as does a thin layer of hot wax of a high melting point when poured into a cold mold.

5. The shrinkage of the positive wax should be so slight as to cause no cracks when it is shrinking against a rigid mold or after it has been mounted.

6. It should become set, yet remain pliable, at a degree of heat low enough for it to be bent or formed with the hands without breaking or cracking the wax or fear of burning the hands; nor should the composition stick to the hands. This pliability is advantageous, since it often becomes necessary to bend a positive composition into a more desirable shape. For example, it may be necessary to bend the positive to relieve it from some undercut surface on a rigid plaster mold. After the positive has been relieved of such an undercut, it can be rebent to its normal position.

These rigid requirements should be kept in mind when considering the published material on positive formulas.

ADVANTAGES OF BEING ABLE TO APPLY THE POSITIVE MATERIAL WITH A BRUSH

A positive wax-resin material that can be brushed, spread, or painted, instead of being poured into the mold, offers decided advantages which may be enumerated as follows:

1. It eliminates the danger of the much dreaded border lines, i.e., lines caused by the junction of hot and cold waxes on a smooth surface. The hot and cold waxes do not blend together, and as a result a line is formed where the waxes of different temperatures meet.

2. The application of the positive with a brush permits a freedom of working without the necessity of lifting heavy molds to turn them in various positions so that the wax will flow evenly and in the same thickness over the entire mold. This same method, when applied to large forms, will prevent a great outlay of physical strength and nerve-racking exertion, which also may require the assistance of helpmates to produce the cast.

3. It is far more economical to "paint" a mold than to pour one, because a quantity of wax may be lost or damaged beyond further use by dripping over the edges of the mold while the latter is being rotated, at the same time soiling one's clothing and burning the hands. The composition may fall on the floor, worktable, or any place except where it should go.

4. "Painting in" makes it possible to fill the separate sections of a piece mold before they are put in place and insures the surfaces of being well and evenly covered. After they have been put in their proper places, the positive pieces may be cemented together by the application of more wax, which may be poured or painted into the cracks between the positive pieces.

5. In painting with the positive substance rather than pouring it into the mold, it is possible to apply masses of different materials and colors next to

one another in the same layer. By the application of one translucent color over another, interesting and lifelike effects may be obtained. One may spread on the mold a wax composition to resemble the skin in color and texture to the edges of a gapping, moist, reddish wound, which would ordinarily glisten with high lights. This wound area of the cast can be painted in with a different material and color from that of the skin. While this second material may have a wax base, it can be incorporated with other materials, such as various gums, to produce the desired glossy effect.

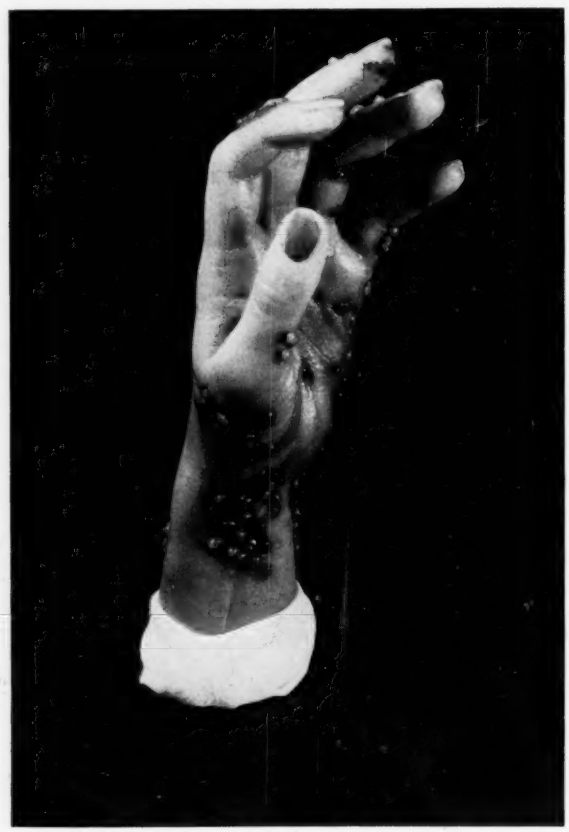

Fig. 1.—A wax-resin moulage of a hand affected with smallpox. This cast was made in a one-piece agar mold. A formula for pouring was used.

6. One may prepare a palette from an ordinary muffin pan and fill each cup with positive wax material which has been mixed with a different color. The caster may then use this muffin pan as an artist does his palette in painting a canvas; the caster paints his mold instead. The thin applications of the various colors on this mold must be strengthened or backed up by a thicker layer of wax, then reinforced with gauze, cheesecloth, or burlap fibers or threads.

In this manner the small blue vessels, which lie beneath the transparent skin, can be incorporated into the cast in the most naturalistic manner. Another indication for the use of color in the same way is a typical hemorrhage under the skin. The fresh flowing blood possesses a different color from stag-

nant blood. Thus the vessels may be painted red on a thin coating of semi-transparent positive formula which has been applied to the mold. These vessel details are then backed with more composition and finally strengthened with gauze, cheesecloth, or burlap. When the cast is finally removed from the mold, the vessels appear to lie under the skin rather than painted on the surface, as is so often done by the caster. It is interesting to note that *red* vessels under an ivory colored skin appear *blue* when viewed through the skin. This may be tested by placing a piece of ivory-colored tracing paper over dark red marks drawn on white paper. These red marks will seem to be blue, provided the right shade of tracing paper and the proper shade of red are used.

Fig. 2.—A wax-resin moulage of a syphilitic gumma. This cast was made in a one-piece agar mold.

There are unlimited possibilities for the specific use of a positive material that can be painted into the mold rather than one that must be poured. In fact, these advantages are too numerous to mention. However, they will suggest themselves as the worker begins the making of molds and casts.

A simple basic formula for brushing into the mold is as follows:

	PARTS BY WEIGHT
Paraffin (about 55° C.)	8
Carnauba wax	1
Rosin, water-white, lump	8
Red oil-soluble dye	q.s.

The following is a suitable formula for pouring into the mold:

Paraffin (about 55° C.)	8
Carnauba wax (light)	1
Rosin, water-white, lump	2
Red oil-soluble dye	q.s.

RESIN*

In spite of the fact that such work is often called "wax" casting, resin is the basic substance in those formulas which are the most simple to use and have the most versatility. Resin gives the compositions toughness and the quality of bending without breaking; it hardens more slowly, and prevents warping and cracking to a great extent. Common rosin or colophony is generally used for this purpose because of its cheapness. Bleached resin (i.e., water-white) serves better where the chromatic intensity is to be kept low. *The resin content should amount to about half of the mixture*, otherwise brushing of the positive mixture into the mold is sometimes rendered difficult and leaves "lap lines" or border lines. Such lines are formed where a hot mixture meets a cold or set mixture of the positive material and causes a line to appear at the junction of the hot and cold mixtures. Compositions containing a large percentage of resin do not flow easily into fine details unless they are painted into the mold.

If the rosin is powdered or if too much is applied, it may oxidize quite readily, turn dark, and separate from the mixture as a nonsoluble precipitate in the bottom of the pot (see Compounding).

WAX

Low Melting Point.—The viscosity and viscidity, or internal friction, of resins are reduced by the addition of a wax. Such waxes are paraffin, white beeswax, ceresin, and spermaceti. Here again the cheaper product, paraffin, is generally chosen.

High Melting Point.—The melting point of a wax and resin composition may be raised by the addition of a wax having a high melting point. Such waxes are carnauba, montan, and Chinese wax. Carnauba, light, is usually selected. The amount of such a wax that is used in the composition should never be more than one-tenth the weight of the resin; otherwise shrinkage and brittleness of the entire composition will result.

In addition to raising the melting point of a wax-resin mixture, carnauba wax also improves the flowing qualities of the mixture and makes it possible to obtain a sharp impression of skin detail, such as the fingerprint pattern. If this wax is not included, the resulting cast may be soft and lack sharp, concise detail.

COLOR

Where it is desirable to have the wax-resin mixture of a basic flesh tone which is composed of red and yellow, a permanent red oil-soluble dye is added. A yellow dye is not used because the yellow of the carnauba wax and resin is sufficient. The red dye mixes with the yellow of the wax and resin to produce the flesh tint. Of course, such a dye must be used sparingly. A gram or so of dye is dissolved first in about 200 c.c. of turpentine. An eye dropper is then

*The term "resin" means an amorphous substance that exudes from plants. The term "rosin" indicates the residue remaining after the distillation of oil of turpentine. Generally, common rosin is the resin of the pine tree. It is also called colophony. Gum mastic, gum dammar, and similar substances are also resins but they are not rosins. *Gum* rosin darkens less rapidly than what is called *wood* rosin. Both are obtained from the pine tree. Gum mastic or gum dammar could be substituted for rosin (colophony) but these resins are decidedly more expensive.

used to place a drop at a time of this solution into the melted wax-resin mixture until the desired shade is reached. From time to time the resulting color may be observed by placing a few drops of the melted mixture on a piece of white paper. When this sets, it is carefully studied in daylight to see whether it is of the basic shade desired. The final monotone wax-resin cast is fixed with shellac or lacquer and then tinted with the proper oil-soluble pigments.

COMPOUNDING

Water is placed in the bottom section of a double boiler and brought to a boil. The fire is then cut down until the water just boils. Rapid boiling may cause the mixture to become too dark. This is important, because excess heat or prolonged heating causes the resin of the mixture to darken and separate as an insoluble resin. The waxes only are placed in the top section and allowed to melt completely before the resin is added. At no time must the pot be allowed to boil vigorously while the resin is mixed with the wax. Heating may be continued with occasional stirring, until the resin is melted completely. Prolonged heating causes it to darken. In fact, some workers prefer to melt the waxes thoroughly and then cut off all heat before adding the resin, depending on the stored-up heat in the waxes and the water section of the double boiler to melt the resin. This does not always suffice, but it will serve to impress upon the worker the importance of not getting the resin too hot.

The basic formulas herewith presented may be varied to suit specific purposes or they may be used as specified. Other waxes and resins may be substituted. Those given were chosen because their cost was decidedly less than waxes and resins having similar qualities, such as melting points and viscosity.

REFERENCES

1. Clarke, C. D., and Cone, Elizabeth S.: Agar Compositions for Molding: The Technique of Compounding and Using, J. Lab. & Clin. Med. **27**: 966, 1942.
2. Woodland, Virginia, Towson, Caroline, and Clarke, C. D.: Rubber Casts of Medical Subjects, J. Lab. & Clin. Med. **26**: 1981, 1941.
3. Clarke, C. D.: Molding and Casting, Baltimore, 1938, John D. Lucas Co.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CIRRHOSIS, Hepatic, Serum Proteins in, Post, J., and Patek, A. J. Arch. Int. Med. 69: 67, 1942; Ibid. 69: 83, 1942.

Of 61 patients with cirrhosis of the liver, 54 had an abnormal albumin-globulin ratio on admission to the hospital. These data confirm the reports of other authors.

The prognosis as to duration of life becomes increasingly grave as the level of serum albumin decreases. The levels of the serum globulin and the serum total protein have no such prognostic significance.

There is a direct correlation between the level of the serum albumin and the clinical course. Clinical improvement is associated with a rise in serum albumin toward normal. In instances of clinical failure there is no sustained rise.

The level of serum albumin is significantly lower in patients with ascites than in those without ascites.

Diuresis is associated with a rise in serum albumin. The mean value for serum albumin at which diuresis occurs is 3.1 Gm. per 100 c.c. (standard deviation, 0.2 Gm.).

From the data presented, it appears that a reduction in serum albumin is an essential factor in the formation of ascites.

Nitrogen balance studies were made on 5 patients with cirrhosis of the liver, ascites, and reduced serum albumin. Although the patients remained in positive nitrogen balance during the periods of high protein feeding, there was no correlated rise in the level of the serum albumin. In this respect these patients differ from persons with simple protein starvation.

The data recorded here indicate that patients with cirrhosis of the liver absorb and retain food protein. The evidence suggests that the mechanism for the synthesis of serum albumin is impaired.

VAGINAL SMEARS, New and Rapid Method of Staining Based Upon a Specific Color Reaction for Glycogen, Mack, H. C. Harper Hosp. Bull. 1: 54, 1942.

1. *Preparation of Smears.*—A moistened cotton applicator is inserted into the vagina and twirled slightly (one complete rotation) against the vaginal wall. The cotton end of the applicator is then rolled lengthwise over the surface of a clean glass slide. By rolling, rather than rubbing, a uniformly thin film of cells, with minimal clumping and cell distortion, results. The film dries almost immediately and may be stained at once.

2. *Staining of Smears.*—Staining is accomplished simply by laying the slide, face down, over a shallow dish containing a small amount of Lugol's solution. Iodine vapors which arise insensibly from the solution suffice to stain the glycogen-containing cells in two or three minutes. Microscopic examination may be carried out immediately. Although such stains fade in from twenty-four to forty-eight hours, restaining (by the same method) may be carried out repeatedly if later examinations are desired.

SYPHILIS, Biologic False Positive Serologic Reactions in Tests for, Mohr, C. F., Moore, J. E., and Eagle, H. Arch. Int. Med. 68: 1161, 1941.

The authors have reported the cases of 11 nonsyphilitic patients with various diseases in whom transitory biologic false positive serologic reactions for syphilis were observed.

When analyzed quantitatively, a low titer was usually revealed in these tests, but in the tests on 2 patients, one with a sore throat and one with pneumonia, a high titer, comparable to that seen in the early stages of syphilis, was observed.

It is suggested that a complement fixation test with spirochetal antigen (pallida test) may be helpful in differentiating false from true positive reactions.

In view of the enormous increase in the routine use of serologic tests for syphilis, the necessity for caution in their interpretation is emphasized.

NOSE, Normal and Abnormal Bacterial Flora of, Jacobson, L. O., and Dick, G. F. J. A. M. A. 117: 2222, 1941.

Cultures of nasal secretion were made as a matter of routine for 500 patients admitted to a general medical service, none of whom had been admitted because of a primary complaint referable to the nose or the paranasal sinuses.

The authors find in agreement with most observers that the normal nasal flora consists chiefly of *Staph. albus* and diphtheroid bacilli, and less frequently, of *Staph. aureus* and *M. catarrhalis.*

Green-forming streptococci and pneumococci are occasional transients in the nasal cavity.

The presence of streptococci, pneumococci, *B. mucosus,* Pfeiffer's bacilli, and diphtheria bacilli indicates disease of the nasal mucosa or sinus disease, or both.

The frequency with which green-forming streptococci, hemolytic streptococci, and pneumococci were isolated by culture of nasal secretion in cases of sinusitis, acute or chronic, shows the diagnostic value of this simple, inexpensive procedure.

ANEMIA, The Significance of Target Cells in, Bohrod, M. G. Am. J. M. Sc. 202: 869, 1941.

Target cells are commonly seen in the regeneration of blood, regardless of the cause of the blood loss.

In acute anemias they are present for only a short time early in the regeneration phase, and they have disappeared by the time a significant rise of erythrocyte count is evident. In chronic anemias they may be present over long periods of time.

The question of the normal shape of the circulating erythrocyte should be re-examined. It is possible, but not yet proved, that the target cell preserves the bowl shape of the intravascular cell in modified form and resists deforming influences which are responsible for producing the biconcave disc seen in blood outside the body.

The target cell is a hyperresistant cell produced by the bone marrow in response to the blood loss. Increased resistance to the hemolytic action of hypotonic saline and to acetic acid has been demonstrated.

The contention that the target cell represents the fundamental defect in Cooley's anemia seems to be unjustified. It is simpler, in the light of available evidence, to regard the target cell in this disease, as in sickle-cell anemia and many other anemias, as a response to the blood destruction rather than the cause of it.

SYPHILIS, The Kahn Verification Test, Chargin, L., and Rein, C. R. Arch. Dermat. & Syph. 44: 1031, 1941.

The reactions to the Kahn verification test were studied for 1,565 persons with various conditions, comprising syphilis, various nonsyphilitic dermatoses, questionable conditions, pregnancy, contagious diseases, pinta, malaria, leprosy, and miscellaneous conditions.

In the group of 349 syphilitic patients, who had received varying amounts of treatment, the verification tests gave the syphilitic type reaction in 100 per cent of those with strongly positive serodiagnostic reactions, in 76.5 per cent of those with weakly positive reactions, and in 40.2 per cent of those showing doubtful reactions. There were no reactions of the syphilitic type in the patients with negative serodiagnostic reactions. The incidence of the general biologic type of verification reaction in the group with negative serologic reactions was 7.1 per cent, and in the group with doubtful serologic reactions it was 12.1 per cent.

In the nonsyphilitic group, 269 in number, there were only 2 patients who had a syphilitic type of verification reaction. Syphilis could not be established in these patients. The incidence of biologic reactions in this group varied from 13.8 to 47.6 per cent.

In the questionable group of 253 members, 83 patients (32.7 per cent) gave a syphilitic type of verification reaction. Further study is necessary to determine whether they actually had syphilis. The incidence of the general biologic (nonsyphilitic) type of reaction in this group varied between 2.3 and 38.6 per cent.

In the group of patients with acute contagious diseases the general biologic (nonsyphilitic) type of verification reaction was obtained in between 29.3 and 64.0 per cent, and in the pregnant women this type reaction was obtained in between 21.2 and 55.5 per cent.

The highest incidence of the general biologic (nonsyphilitic) type of reaction is found to be associated with the doubtful serodiagnostic reactions in all groups.

The percentage of the syphilitic type of verification reaction increases with the increase in the titer of serodiagnostic reaction.

In the group of 268 patients with pinta, all of whom gave strongly positive serodiagnostic reactions, 83.9 per cent gave a syphilitic type of reaction to a verification test.

Agreements as well as discrepancies were observed in a number of blood specimens that were subjected to repeated verification tests.

ZIEHL-NEELSEN STAIN, Sulphonamides and the, Mary John, Sr. Am. J. M. Technol. **7**: 256, 1941.

Observations are reported showing that after the administration of sulfonamide drugs the sputum may contain crystals which are acid-fast and which may rather closely resemble *B. tuberculosis.*

These are seen as long slender, acid-fast rods, sometimes curved, sometimes short and straight, sometimes in bristling clumps.

It is advisable that these drugs be discontinued before sputum examinations for tubercle bacilli are reported as positive.

TRICHINOSIS, A New Precipitin Test for, Roth, H. Acta Path. et microbiol. Scandinav. **18**: 160, 1941.

Living infective larvae of *Trichinella spiralis*, when incubated at 37° C. in the sera of patients suffering from trichinosis or of guinea pigs or rabbits, experimentally trichinized, give rise to the formation in the sera of microscopically visible precipitates. However, no specific deleterious effect of such immune sera on the larvae has as yet been seen. The diagnostic value of this new microscopic test is compared with that of a simplified modification of the usual macroscopic Bachman precipitin test (using a filtrate of the saline extraction of dried trichina larvae as antigen). Both reactions seem to depend on the same antibodies present in immune serum, but the microscopic test may sometimes be more delicate in the demonstration of very slightly developed antibodies.

SPUTUM, Physical and Chemical Properties of, Basch, F. P., Holinger, P., and Poncher, H. G.: Am. J. Dis. Child. **62**: 981, 1941.

The tracheobronchial tree of patients with bronchiectasis contains three essentially different types of sputum, which are not to be confused with the three layers into which the sputum of patients with bronchiectasis or abscess of the lung divides on standing.

To effect the series of the changes which occur spontaneously in the sputum during its movement from the lowest to the highest levels of the tracheobronchial tree, from which it may be coughed out, certain physical, chemical, and bacteriologic actions take place. The changes are not due to physical processes which cause liquefaction or thickening, because a parallel has not been found between the viscosity and the content of solid substances of the sputum.

The secretion from the most dependent portion of the tracheobronchial tree, designated as the third portion, is never coughed up, but is obtainable only by bronchoscopic suction. It has an extremely high viscosity and a high content of organic and inorganic substances. This sputum becomes even thicker after it is removed from the bronchi.

The third portion is moved to a higher level both by the tussive squeeze and by the pressure of secretions to form the second portion, which is found in the trachea and the larger bronchi. The second portion is a liquefied sputum which has a lower viscosity, a lower dried residue and a lower percentage ash than the third portion.

On the top of this secretion, after a rest period, a plug is found which has a moderately high viscosity but a dried residue and percentage of ash which are even lower than those of the second portion.

The sputum of patients with massive collapse of the lung shows a decrease in viscosity during the course of the disease in spite of a marked increase in its content of organic and inorganic material. This spontaneous dilution is probably due to bacterial action.

The characteristic properties of the sputum of patients with bronchiectasis, and postoperative massive collapse of the lung may be due to a variety of factors which may be summarized as follows: (1) selective secretion, (2) selective resorption, (3) speed of movement of secretions from one level to another, (4) bacterial action, and (5) combinations of the foregoing factors.

VAGINAL FLORA in Children, Hardy, G. C. J. Dis. Child. 62: 939, 1941.

In a study in which a limited attempt was made to ascertain the etiologic agents of vaginal infection in girls, the only bacteria which could be incriminated were the gonococcus, the group A streptococcus and, possibly, the diplobacillus of Petit.

In the case of the last two organisms, there is bacteriologic evidence, clinical evidence, or both, of an interrelation between infections of the upper respiratory tract and those of the genital tract.

The streptococcus found simultaneously in the upper respiratory and the vaginal tract were types 11, 24, 25, and 28.

Further study of the diplobacillus, which, superficially at least, may be confused with the gonococcus, is indicated. Gonorrhea should not be diagnosed without complete cultural studies.

Among the patients studied, very few, apart from the premature group, could be considered normal. The large number of purulent secretions found in the children in the hospital wards suggests that such a reaction may be the concomitant of a variety of illnesses or constitutional states. Saprophytic organisms appear to grow in the vaginas of such patients with little restraint. For information concerning normal vaginal flora of childhood the authors recommend study of a group of healthy girls.

More anatomic and histologic studies should provide a better understanding of vaginal infections, so that rational treatment could be devised. An attempt should be made, also, to give adequate clinical attention to patients with such infections as early in the course of the illness as possible.

CARBON DISULFIDE POISONING, Neuropathologic Changes in Experimental, in Cats, Ferraro, A., Jervis, G. A., and Flicker, D. J. Arch. Path. 32: 723, 1941.

Five cats were exposed to varying doses of carbon disulfide. The most important changes in carbon disulfide poisoning consist in (a) diffuse vascular involvement of the productive type, i.e., proliferation of capillaries and hypertrophy of the walls of blood vessels, often leading to endarteritis; and (b) diffuse neurocellular changes, ranging from chromatolysis to severe degeneration, diffusely scattered all over the brain and cerebellum.

Both cortical and subcortical structures are involved. The lesions are particularly evident in the corpora quadrigemina, the cerebellar nuclei, and the vestibular nuclei. The region of the cerebellar nuclei constitutes an area where the change is definitely predominant, and in 4 of 5 cases the pathologic involvement leads to bilateral softening of this region. Next to the cerebellar nuclei, the vestibular area is the one most intensely involved. In an animal in which the exposure to the action of the gas was most prolonged, softening was also found in the lenticular nucleus and in the substantia nigra.

SIMPSON MEMORIAL INSTITUTE
ANN ARBOR, MICH.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

The Diseases of the Eye*

SURELY there are few medical students who have graduated since 1900 who are not well acquainted with May's little textbook. May has always kept it small, although it has perforce become a little larger during the years. A book which is now in its seventeenth American edition, has been translated into nine foreign languages, and has had forty-four editions in foreign lands scarcely requires review.

Infant Nutrition†

THE late Professor Marriott's excellent book has been revised by Dr. P. C. Jeans of the University of Iowa. The work starts with the growth of the infant and child and its physiologic development, particularly as regards nutrition. Then follow sections on the various types of metabolism, carbohydrate, protein, mineral, water, fat, and vitamin. The major portion of the volume is given to gastrointestinal and nutritional disturbances of infancy and childhood, with rather general discussion of remedial measures. General principles are emphasized. There is no padding with sample diets, case histories, and the like.

Diseases of the Digestive System‡

A ROUTINE book covering the field indicated by its title, *Diseases of the Digestive System* is written by fifty well-known contributors. It seems to cover the field adequately and should be of service to gastroenterologists and to men practicing more general medicine.

The Art and Science of Nutrition§

VERY profusely and well illustrated, this volume is written primarily for students of dietetics. It discusses normal nutrition, food requirements under special conditions, dietary therapy, and the choice, preparation, and serving of foods. In the last section, about 140 pages, there is a profusion of tables, recipes, and diets which should be of help to those whose interest is in the scientific preparation of foods.

---

*Manual of the Diseases of the Eye. For Students and General Practitioners. By Charles H. May, M.D., Consulting Ophthalmologist to Bellevue, Mt. Sinai, and French Hospitals, New York. Formerly Chief of Clinic and Instructor in Ophthalmology, Medical Department of Columbia University, and Director of the Eye Service at Bellevue Hospital, New York. Revised with the assistance of Charles A. Perera, M.D., Associate in Ophthalmology, College of Physicians and Surgeons, Medical Department of Columbia University, New York; Assistant Attending Ophthalmologist, Presbyterian Hospital, New York. Cloth, ed. 17, 519 pages, 387 illustrations, including 32 plates and 93 colored figures. William Wood and Company, Baltimore, Md., 1941.

†Infant Nutrition. A Textbook of Infant Feeding for Students and Practitioners of Medicine. By Williams McKim Marriott, B.S., M.D., Late Professor of Pediatrics, Washington University School of Medicine; Physician in Chief, St. Louis Children's Hospital, St. Louis. Revised by P. C. Jeans, A.B., M.D., Professor of Pediatrics, College of Medicine, State University of Iowa, Iowa City. Cloth, ed. 3, 475 pages. The C. V. Mosby Company, St. Louis, Mo., 1941.

‡Diseases of the Digestive System. Edited by Sidney A. Portis, B.S., M.D., F.A.C.P., Associate Professor of Clinical Medicine, Rush Medical College of the University of Chicago; Attending Physician, Michael Reese Hospital; Consulting Physician, Cook County Hospital, Chicago. Cloth, 952 pages, illustrated with 176 engravings, $10.00. Lea & Febiger, Philadelphia, Pa., 1941.

§The Art and Science of Nutrition. A Textbook on the Theory and Application of Nutrition. By Estelle E. Hawley, Ph.D., and Grace Carden, B.S., the University of Rochester, School of Medicine and Dentistry, Rochester, N. Y. Cloth, 619 pages, 140 illustrations, including 12 in color, $3.50. The C. V. Mosby Company, St. Louis, Mo., 1941.

University of California Hospital Formulary*

A POCKET compendium of useful information for internes and fourth-year medical students which would be appropriate for any hospital or any school; this Formulary contains such subjects as prescription writing, materia medica, discussion of buffered and isotonic solutions, dosage tables for various drugs, endocrine products, vitamin preparations, etc. Its therapeutic index suggests the appropriate drug for various symptoms and pathologic states. There are sections on special diets, obstetrical data, laboratory methods, and emergency procedures.

The American and His Food†

A DELIGHTFULLY written history of food habits in the United States from pioneer days, this book presents the difference between menus on the farms and in the cities in early days that is especially interesting. Today, however, the farmer lives better than the city dweller did a century and a half ago.

The improvements in diet which were facilitated by improved transportation facilities rather naturally parallel the advances in commerce in general. The last portion of the book deals with present-day nutrition and needs for the future.

The March of Medicine‡

THIS is the sixth annual compilation of a series of lectures to the laity sponsored by the New York Academy of Medicine. The material covered may be inferred from the list of subjects and their authors: Humanism and Science by Alan Gregg; Paracelsus in the Light of Four Hundred Years by Henry E. Sigerist; Psychiatry and the Normal Life by William Healy; Philosophy as Therapy by Irwin Edman; The Promise of Endocrinology by Oscar Riddle; and What We Do Know About Cancer by Francis Carter Wood.

Essentials of Electrocardiography§

A COMPREHENSIVE discussion of basic principles, technique, and interpretation in the performance of clinical electrocardiography; this book is an excellent guide for students and a reference manual for clinicians.

Diagnostic Procedures and Reagents‖

THIS is a reasonably small book detailing technique for the laboratory diagnosis and control of the communicable diseases, published by the American Public Health Association. It is primarily a book on laboratory methods rather than on control, and as such is authoritative. In addition to the bacterial contagious diseases it covers rickettsial infections, syphilis, rabies, and pathogenic fungi.

The Principal Nervous Pathways¶

THE Principal Nervous Pathways is a splendidly illustrated atlas of the nerve tracts of the brain, cord, and peripheral nerves, including the autonomic nervous system. Each plate has an adequate descriptive legend.

---

*University of California Hospital Formulary and Compendium of Useful Information. Cloth, 270 pages, $2.00. University of California Press, Berkeley and Los Angeles, 1941.

†The American and His Food. A History of Food Habits in the United States. By Richard Osborn Cummings, Assistant Professor of History, Lawrence College. Cloth, 267 pages, $2.50. University of Chicago Press, Chicago, Ill., 1940.

‡The March of Medicine. New York Academy of Medicine. Lectures to the Laity. Cloth, 154 pages, $2.00. Columbia University Press, New York, N. Y., 1941.

§Essentials of Electrocardiography. By Richard Ashman, Ph.D., and Edgar Hull, M.D. Cloth, ed. 2, 373 pages, $5.00. The Macmillan Company, New York, N. Y., 1941.

‖Diagnostic Procedures and Reagents. Technics for the Laboratory Diagnosis and Communicable Diseases. Cloth, 352 pages, $2.75. American Public Health Association, 1790 Broadway, New York, N. Y., 1941.

¶The Principal Nervous Pathways—Neurological Charts and Schemas With Explanatory Notes. By Andrew Theodore Rasmussen, Ph.D., Professor of Neurology, Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minn. Cloth, ed. 2, 73 pages. The Macmillan Company, New York, N. Y., 1941.

Physical Medicine*

THERE have been many volumes on physiotherapy. This is the first the reviewer has seen that appears to be fully authentic and free of overenthusiasm for one phase or another of physical therapy. It describes the methods of physical therapy as they are used at the Mayo Clinic. No section appears to receive undue emphasis. The author describes the application of heat and cold, light therapy, electrotherapy, diathermy, hydrotherapy, and mechanotherapy. A section deals with the application of the principles of physical therapy to various special diseases. Another section deals with the organization of physical therapy in hospitals. The introductory portion, which deals with the history of physical therapy, is especially interesting.

This volume will be useful not only to physiotherapists, but to others who wish to know the desirability of physical measures in special cases.

Immunity Against Animal Parasites†

THE first volume dealing with this phase of medical study was Taliaferro's *Immunology of Parasitic Infections*, published in 1929. Culbertson's book takes up where Taliaferro left off, bringing the studies up to date. Part I deals with natural resistance and acquired immunity. Part II discusses immunity in specific diseases, such as amoebiases, leishmaniases, trypanosomiases, malaria, coccidioses, trematodiases, cestodiases, nematodiases. The third section is on applied immunology; it classifies parasites and discusses preventive vaccination and diagnostic procedures.

Students of bacterial immunity will find much of interest in the volume. There is also much of practical service to clinicians.

Pathology of the Oral Cavity‡

WRITTEN primarily for the dentist, this volume also contains sections on diseases of the jawbones and the oral mucosa. It is abundantly and well illustrated. All sections are short and concise. Emphasis is on diagnosis and significance of the lesions rather than upon therapeutic procedures.

Practical Methods in Biochemistry§

A STUDENTS' laboratory manual used in the department of biochemistry at the University of Chicago. Although it will find its greatest use in student laboratory instruction, it will undoubtedly also serve well as a laboratory reference manual since it contains detailed instructions for the technique of the various clinical laboratory procedures. Especially valuable for reference purposes is the appendix in which fifty pages of small type are given to the preparation of standard reagents and dilutions.

---

*Physical Medicine. The Employment of Physical Agents for Diagnosis and Therapy. By Frank H. Krusen, M.D., F.A.C.P., Associate Professor of Physical Medicine, the Mayo Foundation, University of Minnesota; Head of the Section on Physical Therapy, the Mayo Clinic; Member of the Council on Physical Therapy of the American Medical Association; Past President of the American Congress of Physical Therapy; Past President of the Academy of Physical Medicine. Cloth, 845 pages, with 351 illustrations. W. B. Saunders Company, Philadelphia and London, 1941.

†Immunity Against Animal Parasites. By James T. Culbertson, Assistant Professor of Bacteriology, College of Physicians and Surgeons, Columbia University. Cloth, 274 pages, $3.50. Columbia University Press, New York, N. Y., 1941.

‡Pathology of the Oral Cavity. By Lester Richard Cahn, D.D.S., Associate Professor of Dentistry (Oral Pathology), Columbia University, Fellow of the American Association for the Advancement of Science. Fellow of the New York Academy of Dentistry. Associate Fellow of the New York Academy of Medicine. The Williams & Wilkins Company, Baltimore, Md., 1941.

§Practical Methods in Biochemistry. By Frederick C. Koch, Frank P. Hixon Distinguished Service Professor of Biochemistry, University of Chicago. Cloth, ed. 3 (revised), 314 pages. A William Wood Book. The Williams & Wilkins Company, Baltimore, Md., 1941.